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(54) Neuer metabotroper-Rezeptor-Komplex aus dem zentralen Nervensystem

(57) Die vorliegende Erfindung betrifft isolierte Proteine, die für neue metabotrope GABA-Rezeptoren kodieren und mit $GABA_B$-Rezeptorproteinen einen neuen metabotroper GABA-Rezeptor-Komplex (= Proteinheteromer) bilden, und Nukleinsäuresequenzen oder rekombinante Nukleinsäurekonstrukte, die für die Proteine kodieren.
Die Erfindung betrifft außerdem Wirtsorganismen oder transgene Tiere, enthaltend die Nukleinsäureseuqnenzen oder die rekombinanten Nukleinsäurekonstrukte sowie mono- oder polyklonale Antikörper, die gegen die isolierten Proteine gerichtet sind.
Weiterhin betrifft die Erfindung ein Verfahren zum Auffinden von Substanzen mit spezifischer Bindungsaffinität an die erfindungsgemäßen neuen GABA-Rezeptoren bzw. die erfindungsgemäßen GABA-Rezeptor-Komplexe, ein Verfahren zum qualitativen oder quantitativen Nachweis der erfindungsgemäßen Nukleinsäuresequenzen oder der erfindungsgemäßen Proteine sowie einem Verfahren zum Auffinden von Substanzen, die spezifisch an einen erfindungsgemäßen GABA-Rezeptor oder an eine erfindungsgemäße Nukleinsäuresequenz binden. Ferner betrifft die Erfindung die Verwendung der Nukleinsäuresequenzen und Proteine.

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Beschreibung

Die vorliegende Erfindung betrifft isolierte Proteine, die für neue metabotrope GABA-Rezeptoren kodieren und mit $GABA_B$-Rezeptorproteinen einen neuen metabotroper GABA-Rezeptor-Komplex (= Proteinheteromer) bilden, und Nukleinsäuresequenzen oder rekombinante Nukleinsäurekonstrukte, die für die Proteine kodieren.

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Weiterhin betrifft die Erfindung ein Verfahren zum Auffinden von Substanzen mit spezifischer Bindungsaffinität an die erfindungsgemäßen neuen GABA-Rezeptoren bzw. die erfindungsgemäßen GABA-Rezeptor-Komplexe, ein Verfahren zum qualitativen oder quantitativen Nachweis der erfindungsgemäßen Nukleinsäuresequenzen oder der erfindungsgemäßen Proteine sowie einem Verfahren zum Auffinden von Substanzen, die spezifisch an einen erfindungsgemäßen GABA-Rezeptor oder an eine erfindungsgemäße Nukleinsäuresequenz binden. Ferner betrifft die Erfindung die Verwendung der Nukleinsäuresequenzen und Proteine.

Im zentralen Nervensystem von Vertebraten wird vornehmlich γ-Amino-Buttersäure (GABA) als inhibierender Neurotransmitter verwendet. GABA interagiert mit zwei Rezeptortypen $GABA_A$ und $GABA_B$. Gut charakterisiert ist die Wirkung von GABA auf ionotrope Rezeptoren, die $GABA_A$-Rezeptoren (Barnard et al., Trends Neurosci., 10, 1987: 502–509). Diese heteromeren Komplexe bilden Anionenkanäle, die auf Ligandenbindung hin geöffnet werden. Die Ausschüttung von GABA führt über die Aktivierung dieser Kanäle zu einem Chloridioneneinstrom, einem inhibitorischen postsynaptischen Strom, in die Zelle. $GABA_A$-Rezeptoren sind die Angriffspunkte einer Reihe von Medikamenten wie beispielsweise von Benzodiazepinen, Barbituraten und anderen mehr [North (ed), 1994, Ligand- and Voltage Gated Ion Channels, in: Handbook of receptors and channels, vol. 2, CRC Press, Inc. and Smith and Olson, Trends Neurosci., 16, 1995: 162–168].

1981 wurden zum ersten Mal Bindungsstellen für GABA nachgewiesen, die unabhängig von Bindungsstellen auf $GABA_A$-Rezeptoren vorliegen (Hill and Bowery, Nature, 290, 1981: 149–152). Sie befinden sich auf GABA-Rezeptoren, die intrazellulär an G-Proteine gekoppelt sind. Über diese G-Proteine sind diese Rezeptoren an neuronale Kalium- und Calciumkanäle gekoppelt. Diese neuen GABA-Rezeptoren (= $GABA_B$-Rezeptoren) werden auch als metabotrope GABA-Rezeptoren bezeichnet. Diese $GABA_B$-Rezeptoren sind über das zentrale und periphäre Nervensystem verteilt (Ong et al., Life Sciences, Vol. 46, 1990: 1489–1501, Bowery et al., Drug Res., 42 (1), 1992: 215–223). Diese Rezeptoren werden sowohl prä- als auch postsynaptisch gefunden. An der Präsynapse kontrollieren die metabotropen GABA-Rezeptoren (= $GABA_B$-Rezeptoren) die Ausschüttung verschiedener Neurotransmitter wie GABA, L-Glutamat, Noradrenalin, Dopamin, Serotonin, Substanz P, Cholecystokinin, Somatostatin und anderen. Liganden, Agonisten oder Antagonisten für $GABA_B$-Rezeptoren, die die Ausschüttung eines spezifischen Neurotransmitters oder Neuropeptides regulieren, können benutzt werden, um Ungleichgewichte zwischen verschiedenen Neurotransmittersystemen, wie sie bei neurodegenerativen Erkrankungen, exzitotoxischen Begleiterscheinungen von neurologischen Erkrankungen sowie bei psychiatrischen Krankheiten vorkommen, auszugleichen. An der Postsynapse findet man $GABA_B$-Rezeptoren, die über Gi-Proteine verschiedene Kalium-Kanäle aktivieren. Null-Mutationen für einen solchen Kanal in transgenen Mäusen führt zum Verlust der durch GABA bewirkten späten Inhibierung und dadurch zu spontanen Krämpfen. $GABA_B$-Rezeptoren sind an Änderungen der synaptischen Effizienz beteiligt, die Lern- und Gedächtnisvorgängen zugrundeliegen. $GABA_B$-Rezeptor-Agonisten zeigen positive Wirkung in Tiermodellen für chronische Schmerzen sowie Kokain-Abhängigkeit. Antagonisten wirken sich positiv in Modellen von "Absence-Epilepsie" aus (Bettler et al., Curr. Opin. Neurolbiol., 8, 1998: 345–350). Aktivierung von $GABA_B$-Rezeptoren dämpft übererregte neuronale Verknüpfungen. $GABA_B$-Rezeptoren sind daher geeignete molekulare Targets für die Behandlung von Epilepsie, Schlaganfall, kognitiven Verlusten, chronischen Schmerzen und weiteren neurologischen Erkrankungen sowie für die Behandlung von psychischen Erkrankungen wie Angst, depressiven Erkrankungen, Schizophrenie, Migräne und anderen. Sie sind auch als Targets für die Therapie von Kokain-Abhängigen sowie als Angriffspunkt für neurartige kognitive Enhancer geeignet. Ein $GABA_B$-Rezeptor-Agonist, Baclofen (Lioresal), wird für die Behandlung von multipler Sklerose und bei den Folgen von Rückenmarksverletzungen klinisch eingesetzt (Bowery, Annu. Rev. Pharmacol. Toxicol., 33, 1993: 109–147).

Auch im peripheren Nervensystem (PNS) werden positive Effekte von $GABA_B$-Rezeptor-Agonisten vermutet, beispielsweise bei Entzündungen und Atemwegserkrankungen.

Einzelne biochemische und pharmakologische Erkenntnisse können möglicherweise dahingehend interpretiert werden, daß im zentralen Nervensystem mehrere $GABA_B$-Rezeptor-Subtypen mit verschiedenen Funktionen existieren. Bisher wurde die cDNA für einen $GABA_B$-Rezeptor gefunden, der in zwei aminoterminalen Spleissformen auftritt, welche sich pharmakologisch kaum unterscheiden (Kaupmann et al., Nature, 386, 239–246, 1997, WO97/46675). Dieser $GABA_B$-Rezeptor weist nach Expression in heterologen Systemen ähnlich hohe Affinitäten zu den bekannten $GABA_B$-Rezeptor-Antagonisten auf, wie sie im Gehirn gefunden werden. Die Affinitäten von $GABA_B$-Rezeptor-Agonisten zu diesem klonierten $GABA_B$-Rezeptor liegen dagegen etwa um den Faktor 100 unter den im Gehirn gemessenen Werten (Kaupmann et al., Nature, 386, 239–246, 1997). Verschiedene $GABA_B$-Rezeptorantagonisten und -agonisten und ihre Wirkung sind Froestl et al., J. Med. Chem. Vol. 38, 1995: 3297–3312 und 3313–3331 zu entnehmen.

Da $GABA_B$-Rezeptoren eine zentrale Rolle bei verschiedenen pathologischen Prozessen des zentralen und peripheren Nervensystems spielen bzw. an derartigen Prozessen beteiligt sind, sind sie gesuchte Targets für die Entwicklung neuer Pharmaka.

Es bestand daher die Aufgabe neue $GABA_B$-Rezeptoren oder Proteine, die mit den $GABA_B$-Rezeptoren interagieren, zu identifizieren und zu charakterisieren, die möglichst hochaffine Bindungsstellen für $GABA_B$-Rezeptor-Agonisten und -Antagonisten besitzen und die damit die Entwicklung molekularer Testsysteme ermöglichen, mit denen man viele Tausend verschiedene Verbindungen nach hochaffinen Substanzen in kurzer Zeit durchsuchen kann. Diese so charakterisierten und spezifisch am $GABA_B$-Rezeptor angreifenden Substanzen sind potentielle Kandidaten für Wirkstoffe gegen Krankheiten wie Epilepsie, Schlaganfall, psychische Erkrankungen wie Angst, manischdepressive Erkrankungen, Schi-

zophrenie, Migräne und andere mehr.

Diese Aufgabe wurde mit dem erfindungsgemäßen Proteinheteromer, enthaltend mindestens ein $GABA_B$-Rezeptorprotein und mindestens ein Protein mit der in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellte Aminosäuresequenz oder eine daraus durch Substitution, Inversion, Insertion oder Deletion von einem oder mehreren Aminosäureresten erhältliche Sequenz, wobei wenigstens noch eine der wesentlichen biologischen Eigenschaften des in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Proteins oder des Proteinheteromer erhalten bleibt bzw. mit dem isolierten Protein, enthaltend die in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellte Aminosäuresequenz oder eine daraus durch Substitution, Inversion, Insertion oder Deletion von einem oder mehreren Aminosäureresten erhältliche Sequenz, wobei wenigstens noch eine der wesentlichen biologischen Eigenschaften des in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Proteins erhalten bleibt, gelöst.

Unter den erfindungsgemäßen Proteinheteromeren sind GABA-Rezeptor-Komplexe vorteilhaft metabotrope GABA-Rezeptor-Komplexe enthaltend mindestens einen $GABA_B$-Rezeptorprotein und mindestens ein Protein mit der in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Aminosäuresequenz zu verstehen. In WO97/46675 werden geeignete $GABA_B$-Rezeptorproteine, die vorteilhaft in den Proteinheteromeren enthalten sein können, beschrieben. Diese GABAB-Rezeptorproteine tragen in WO97/46675 die Sequenzbezeichnungen bzw. Klonnamen "SEQ ID NO: 1" bzw. "GABABR1a rat" (kloniert aus Rattus norvegicus), "SEQ ID NO: 3" bzw. "GABABR1a/b human" (kloniert aus Homo sapiens), "SEQ ID NO: 5" bzw. "GABABR1b rat" (kloniert aus Rattus norvegicus) und "SEQ ID NO: 7" bzw. "GABABR1b human" (kloniert aus Homo sapiens). Auf diese Rezeptoren und die Schrift WO97/46675 wird an dieser Stelle ausdrücklich bezug genommen.

Unter den erfindungsgemäßen isolierten Proteinen sind Proteine zu verstehen, die eine in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellte Aminosäuresequenz oder eine daraus durch Substitution, Inversion, Insertion oder Deletion von einem oder mehreren Aminosäureresten erhältliche Sequenz enthalten, wobei wenigstens noch eine der wesentlichen biologischen Eigenschaften des in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Proteins erhalten bleibt. Dabei können beispielsweise bestimmte Aminosäuren durch solche mit ähnlichen physikochemischen Eigenschaften (Raumerfüllung, Basizität, Hydrophobizität etc.) ersetzt werden. Beispielsweise werden Argininreste gegen Lysinreste, Valinreste gegen Isoleucinreste oder Asparaginsäurereste gegen Glutaminsäurereste ausgetauscht. Es können aber auch ein oder mehrere Aminosäuren in ihrer Reihenfolge vertauscht, hinzugefügt oder entfernt werden, oder es können mehrere dieser Maßnahmen miteinander kombiniert werden. Die solchermaßen gegenüber der SEQ ID NO: 2 oder SEQ ID NO: 4 veränderten Proteine besitzen wenigstens 60%, bevorzugt wenigstens 70% und besonders bevorzugt wenigstens 90% Sequenzidentität zu den Sequenzen SEQ ID NO: 2 oder SEQ ID NO: 4 berechnet nach dem Algorithmus von "Altschul et al., J. Mol. Biol., 215, 403–410, 1990".

Unter der wesentlichen biologischen Eigenschaft der erfindungsgemäßen Proteine bzw. Proteinheteromere sind der oder die transmembranen Bereiche, der aminoterminale Bereich und wesentlich der carboxyterminale Bereich des Proteins allein oder im Proteinheteromer zu verstehen (siehe **Fig. 2**). Diese Proteinbereiche ermöglichen die spezielle biologische Wirkung der Proteine bzw. Proteinheteromere. Diese wesentlichen biologischen Eigenschaften beinhalten außerdem die hochaffine Bindung (Kd<10nM) spezifischer synthetischer oder natürlicher Agonisten und Antagonisten an die erfindungsgemäßen Proteine mit der in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellte Aminosäuresequenz, die Signalweiterleitung an ein intrazelluläres G-Protein sowie die Interaktion mit den oben genannten, bekannten $GABA_B$-Rezeptoren.

Das isolierte Protein und seine funktionellen Varianten lassen sich vorteilhafterweise aus dem Gehirn von Mammalia wie Homo sapiens oder Rattus norvegicus isolieren. Auch Homologe aus anderen Mammalia sind unter funktionellen Varianten zu verstehen.

Ein weiterer Gegenstand der Erfindung sind Nukleinsäuresequenzen, die für die oben beschriebenen Proteine kodieren, insbesondere für solche mit der in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Primärstruktur. Die Nukleinsäuresequenz aus Rattus norvegicus oder Homo sapiens ist in SEQ ID NO: 1 bzw. in SEQ ID NO: 3 dargestellt.

Nach Isolierung und Sequenzierung sind die erfindungsgemäßen Nukleotidsequenzen SEQ ID NO: 1 und SEQ ID NO: 3 oder deren funktionelle Äquivalente wie z. B. Allelvarianten erhältlich. Unter Allelvarianten sind SEQ ID NO: 1- oder SEQ ID NO: 3-Varianten zu verstehen, die 60 bis 100% Homologie auf Aminosäureebene, bevorzugt 70 bis 100%, ganz besonders bevorzugt 90 bis 100% aufweisen. Allelvarianten umfassen insbesondere solche funktionellen Varianten, die durch Deletion, Insertion oder Substitution von Nukleotiden aus der in SEQ ID NO: 1 oder SEQ ID NO: 3 dargestellten Sequenz erhältlich sind, wobei wenigstens noch eine der wesentlichen biologischen Eigenschaften erhalten bleibt. Homologe oder sequenzverwandte Nukleinsäuresequenzen können aus allen Säugerspezies einschließlich Mensch nach gängigen Verfahren durch Homologiescreening durch Hybridisierung mit einer Probe der erfindungsgemäßen Nukleinsäuresequenzen oder Teilen davon isoliert werden.

Unter funktionellen Äquivalenten sind auch Homologe von SEQ ID NO: 1 oder SEQ ID NO: 3 beispielsweise ihre Homologen aus anderen Mammalia, verkürzte Sequenzen, Einzelstrang-DNA oder RNA der codierenden und nichtcodierenden DNA-Sequenz zu verstehen.

Solche funktionellen Äquivalente lassen sich ausgehend von den in SEQ ID NO: 1 oder SEQ ID NO: 3 beschriebenen DNA-Sequenzen oder Teilen dieser Sequenzen, beispielsweise mit üblichen Hybridisierungsverfahren oder der PCR-Technik aus anderen Vertebraten wie Mammalia isolieren. Diese DNA-Sequenzen hybridisieren unter Standardbedingungen mit den erfindungsgemäßen Sequenzen. Zur Hybrisierung werden vorteilhaft kurze Oligonukleotide der konservierten Bereiche beispielsweise aus den Transmembranbereichen oder aus dem aminoterminalen Bereich, die über Vergleiche mit anderen Transmembranproteinen speziell anderen GABA-Rezeptoren in dem Fachmann bekannter Weise ermittelt werden können, verwendet. Es können aber auch längere Fragmente der erfindungsgemäßen Nukleinsäuren oder die vollständigen Sequenzen für die Hybridisierung verwendet werden. Je nach der verwendeten Nukleinsäure Oligonukleotid, längeres Fragment oder vollständige Sequenz oder je nachdem welche Nukleinsäureart DNA oder RNA für die Hybridisierung verwendet werden, varieren diese Standardbedingungen. So liegen beispielsweise die Schmelztemperaturen für DNA : DNA-Hybride ca 10°C niedriger als die von DNA : RNA-Hybriden gleicher Länge.

Unter Standardbedingungen sind beispielsweise je nach Nukleinsäure Temperaturen zwischen 42 und 58°C in einer wäßrigen Pufferlösung mit einer Konzentration zwischen 0,1 bis 5 × SSC (1 × SSC = 0,15 M NaCl, 15 mM Natriumcitrat, pH 7,2) oder zusätzlich in Gegenwart von 50% Formamid wie beispielsweise 42°C in 5 × SSC, 50% Formamid zu verstehen. Vorteilhafterweise liegen die Hybridisierungsbedingungen für DNA : DNA-Hybride bei 0,1 × SSC und Temperaturen zwischen etwa 20°C bis 45°C, bevorzugt zwischen etwa 30°C bis 45°C. Für DNA : RNA-Hybride liegen die Hybridisierungsbedingungen vorteilhaft bei 0,1 × SSC und Temperaturen zwischen etwa 30°C bis 55°C, bevorzugt zwischen etwa 45°C bis 55°C. Diese angegebenen Temperaturen für die Hybridisierung sind beispielhaft kalkulierte Schmelztemperaturwerte für eine Nukleinsäure mit einer Länge von ca. 100 Nukleotiden und einem G + C-Gehalt von 50% in Abwesenheit von Formamid. Die experimentellen Bedingungen für die DNA-Hybridisierung sind in einschlägigen Lehrbüchern der Genetik wie beispielsweise Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989, beschrieben und lassen sich nach dem Fachmann bekannten Formeln beispielsweise abhängig von der Länge der Nukleinsäuren, der Art der Hybride oder dem G + C-Gehalt berechnen. Weitere Informationen zur Hybridisierung kann der Fachmann folgenden Lehrbüchern entnehmen: Ausubel et al. (eds), 1998, Current Protocols in Molecular Biology, John Wiley & Sons, New York; Hames and Higgins (eds), 1985, Nucleic Acids Hybridization: A Practical Approach, IRL Press at Oxford University Press, Oxford; Brown (ed), 1991, Essential Molecular Biology: A Practical Approach, IRL Press at Oxford University Press, Oxford.

Außerdem sind unter Homologe der Sequenzen SEQ ID NO: 1 und SEQ ID NO: 3 Derivate wie beispielsweise Promotorvarianten zu verstehen. Die Promotoren, die den angegebenen Nukleotidsequenzen gemeinsam oder einzeln vorgeschaltet sind, können durch ein oder mehrere Nukleotidaustausche, durch Insertion(en) und/oder Deletion(en) verändert sein, ohne daß aber die Funktionalität bzw. Wirksamkeit der Promotoren beeinträchtigt sind. Des weiteren können die Promotoren durch Veränderung ihrer Sequenz in ihrer Wirksamkeit erhöht oder komplett durch wirksamere Promotoren auch artfremder Organismen ausgetauscht werden.

Unter Derivaten sind auch vorteilhaft Varianten zu verstehen, deren Nukleotidsequenz im Bereich −1 bis −1000 vor dem Startkodon so verändert wurden, daß die Genexpression und/oder die Proteinexpression verändert, bevorzugt erhöht wird. Weiterhin sind unter Derivaten auch Varianten zu verstehen, die am 3'-Ende verändert wurden.

Für eine optimale Expression heterologer Gene in Organismen ist es vorteilhaft die Nukleinsäuresequenzen entsprechend des im Organismus verwendeten spezifischen "codon usage" zu verändern. Der "codon usage" läßt sich anhand von Computerauswertungen anderer, bekannter Gene des betreffenden Organismus leicht ermitteln.

Weiterhin ist es von Vorteil die erfindungsgemäßen Nukleinsäuren SEQ ID NO: 1 oder SEQ ID NO: 3 allein oder die Nukleinsäuren SEQ ID NO: 1 oder SEQ ID NO: 3 und eine Sequenz, die für einen $GABA_B$-Rezeptorprotein kodiert, funktionell mit mindestens einem genetischen Regulationselement zu den erfindungsgemäßen rekombinanten Nukleinsäurekonstrukten zu verknüpfen.

Dazu werden die erfindungsgemäßen Nukleinsäuresequenzen üblicherweise mit genetischen Regulationselementen wie Transkriptions- und Translationssignalen funktionell verknüpft. Diese Verknüpfung kann je nach gewünschter Anwendung zu einer Erhöhung oder Erniedrigung der Genexpression führen. Mit den solchermaßen hergestellten rekombinanten Nukleinsäurekonstrukten werden anschließend Wirtsorganismen transformiert. Zusätzlich zu diesen neuen Regulationssequenzen kann die natürliche Regulation dieser Sequenzen vor den eigentlichen Strukturgenen noch vorhanden sein und gegebenenfalls genetisch verändert worden sein, so daß die natürliche Regulation ausgeschaltet und die Expression der Gene erhöht wurde. Das Genkonstrukt kann aber auch einfacher aufgebaut sein, das heißt es werden keine zusätzlichen Regulationssignale vor die Sequenzen inseriert und der natürliche Promotor mit seiner Regulation wird nicht entfernt. Stattdessen wird die natürliche Regulationssequenz so mutiert, daß keine Regulation mehr erfolgt und die Genexpression gesteigert wird. Auch am 3'-Ende der Nukleinsäure-Sequenzen können zusätzliche vorteilhafte regulatorische Elemente inseriert werden. Die Nukleinsäuresequenzen für die Sequenzen SEQ ID NO: 1 oder SEQ ID NO: 3 und/oder für die $GABA_B$-Rezeptorproteine können in einer oder mehreren Kopien im Genkonstrukt enthalten sein, oder auf getrennten Genkonstrukten lokalisiert sein.

Vorteilhafte Regulationssequenzen für das erfindungsgemäße Verfahren sind beispielsweise in Promotoren wie cos-, tac-, trp-, tet-, trp-tet-, lpp-, lac-, lpp-lac-, lacIq-, T7-, T5-, T3-, gal-, trc-, ara-, SP6-, 1-PR- oder im 1-PL-Promotor enthalten, die vorteilhafterweise in gram-negativen Bakterien Anwendung finden. Weitere vorteilhafte Regulationssequenzen sind beispielsweise in den gram-positiven Promotoren wie amy und SPO2, in den Hefepromotoren wie ADC1, MFa, AC, P-60, CYC1, GAPDH oder in Mammaliapromotoren wie CaM-KinaseII, CMV, Nestin, L7, BDNF, NF, MBP, NSE, β-Globin, GFAP, GAP43, Tyrosin Hydroxylase, Kainat-Rezeptor-Untereinheit 1, Glutamat-Rezeptor-Untereinheit B enthalten.

Prinzipiell können alle natürlichen Promotoren mit ihren Regulationssequenzen wie die oben genannten verwendet werden. Darüberhinaus können auch synthetische Promotoren vorteilhaft verwendet werden.

Diese regulatorischen Sequenzen sollen die gezielte Expression der Nukleinsäuresequenzen und der Proteinexpression ermöglichen. Dies kann beispielsweise je nach Wirtsorganismus bedeuten, daß das Gen erst nach Induktion exprimiert oder überexprimiert wird, oder daß es sofort exprimiert und/oder überexprimiert wird.

Die regulatorischen Sequenzen bzw. Faktoren können dabei vorzugsweise die Expression positiv beeinflussen und dadurch erhöhen. So kann eine Verstärkung der regulatorischen Elemente vorteilhafterweise auf der Transkriptionsebene erfolgen, indem starke Transkriptionssignale wie Promotoren und/oder "Enhancer" verwendet werden. Daneben ist aber auch eine Verstärkung der Translation möglich, indem beispielsweise die Stabilität der mRNA verbessert wird.

Unter "Enhancer" sind beispielsweise DNA-Sequenzen zu verstehen, die über eine verbesserte Wechselwirkung zwischen RNA-POlymerase und DNA eine erhöhte Expression bewirken. Als weitere Regulationssequenzen seien beispielhaft die locus control regions, silencer oder jeweilige Teilsequenzen davon genannt. Diese Sequenzen können vorteilhaft für eine gewebespezifische Expression verwendet werden.

Eine bevorzugte Ausführungsform ist die Verknüpfung der erfindungsgemäßen Nukleinsäuresequenz mit einem Promotor, wobei der Promotor 5' up stream zu liegen kommt. Weitere Regulationssignale wie 3' gelegene Terminatoren oder Polyadenylierungssignale oder Enhancer können funktionell in dem Nukleinsäurekonstrukt Anwendung finden.

Unter dem Begriff des erfindungsgemäßen "rekombinanten Nukleinsäurekonstrukts bzw. Genkonstrukts" sind auch komplette Vektorkonstrukte zu verstehen. Diese Vektorkonstrukte oder Vektoren werden zur Expression in einem geeigneten Wirtsorganismus verwendet. Vorteilhafterweise werden die erfindungsgemäßen Nukleinsäuren und/oder die Gene für die $GABA_B$-Rezeptoren in einen wirtsspezifischen Vektor inseriert, der eine optimale Expression der Gene im ausgesuchten Wirt ermöglicht. Vektoren sind dem Fachmann wohl bekannt und können beispielsweise aus dem Buch Cloning Vectors (Eds. Pouwels P. H. et al. Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018) entnommen werden. Unter Vektoren sind außer Plasmiden auch alle anderen dem Fachmann bekannten Vektoren wie beispielsweise Phagen, Viren wie SV40, CMV, Baculovirus, Adenovirus, Transposons, IS-Elemente, Phasmide, Phagemide, Cosmide, lineare oder zirkuläre DNA zu verstehen. Diese Vektoren können autonom im Wirtsorganismus repliziert oder chromosomal repliziert werden. Für die Integration in Mammalia wird vorteilhaft lineare DNA verwendet.

Die Expression der erfindungsgemäßen Nukleinsäuresequenzen bzw. des rekombinanten Nukleinsäurekonstrukts kann vorteilhaft durch Erhöhen der Genkopienzahl und/oder durch Verstärkung regulatorischer Faktoren, die die Genexpression positiv beeinflussen, erhöht werden. So kann eine Verstärkung regulatorischer Elemente vorzugsweise auf der Transkriptionsebene erfolgen, indem stärkere Transkriptionssignale wie Promotoren und Enhancer verwendet werden. Daneben ist aber auch eine Verstärkung der Translation möglich, indem beispielsweise die Stabilität der mRNA verbessert, oder die Ableseeffizienz dieser mRNA an den Ribosomen erhöht wird.

Zur Erhöhung der Genkopienzahl können die Nukleinsäuresequenzen oder homologe Gene, beispielsweise in ein Nukleinsäurefragment bzw. in einen Vektor eingebaut werden, der vorzugsweise die den jeweiligen Genen zugeordnete, regulatorische Gensequenzen oder analog wirkende Promotoraktivität enthält. Insbesondere werden solche regulatorische Sequenzen verwendet, die die Genexpression verstärken.

Die erfindungsgemäßen Nukleinsäuresequenzen können zusammen mit den für die $GABA_B$-Rezeptoren kodierenden Sequenzen in einen einzelnen Vektor kloniert werden und anschließend in dem gewünschten Organismus exprimiert werden. Alternativ kann auch jede der beschriebenen Nukleinsäuresequenzen und die für die $GABA_B$-Rezeptoren kodierenden Sequenzen in je einen einzelnen Vektor gebracht und diese getrennt in den jeweiligen Organismus über übliche Methoden wie Transformation, Transfektion, Transduktion, Elektrophoration oder Partikel-Gun verbracht werden.

Darüberhinaus kann das erfindungsgemäße Nukleinsäurekonstrukt oder die erfinungsgemäßen Nukleinsäuren auch in Form therapeutisch oder diagnostisch geeigneter Fragmente exprimiert werden. Zur Generierung des rekombinanten Proteins können Vektorsysteme oder Oligonukleotide verwendet werden, die die Nukleinsäuren oder das Nukleinsäurekonstrukt um bestimmte Nukleotidsequenzen verlängern und damit für veränderte Polypeptide kodieren, die einer einfacheren Reinigung dienen. Als solche "Tags" sind in der Literatur z. B. Hexa-Histidin-Anker bekannt oder Epitope, die als Antigene verschiedener Antikörper erkannt werden können (Studier et al., Meth. Enzymol., 185, 1990: 60–89 und Ausubel et al. [eds.]m 1998, Current Protocols in Molecular Biology, John Wiley & Sons, New York).

Als Wirtsorganismen sind prinzipiell alle Organismen geeignet, die eine Expression der erfindungsgemäßen Nukleinsäuren, ihrer Allelvarianten, ihrer funktionellen Äquivalente oder Derivate oder des rekombinanten Nukleinsäurekonstrukt allein oder zusammen mit einer Sequenz, die für $GABA_B$-Rezeptorproteine kodiert, ermöglichen. Unter Wirtsorganismen sind beispielsweise Bakterien, Pilze, Hefen, pflanzliche oder tierische Zellen zu verstehen. Bevorzugte Organismen sind Bakterien wie Escherichia coli, Streptomyces, Bacillus oder Pseudomonas, eukaryotische Mikroorganismen wie Saccharomyces cerevisiae, Aspergillus, höhere eukaryotische Zellen aus Mensch oder Tier, beispielsweise COS-, Hela-, HEK293-, Sf9- oder CHO-Zellen.

Gewünschtenfalls kann das Genprodukt auch in transgenen Organismen wie transgenen Tieren z. B. Mäusen, Ratten, Schafen, Rindern oder Schweinen zur Expression gebracht werden. Auch transgene Pflanzen sind im Prinzip denkbar. Bei den transgenen Organismen kann es sich auch um sogenannte Knock-Out Tiere handeln.

Dabei können die transgenen Tiere eine funktionelle oder nicht funktionelle erfindungsgemäße Nukleinsäuresequenz oder ein funktionelles oder nicht funktionelles Nukleinsäurekonstrukt allein oder in Kombination mit einer funktionellen oder nicht funktionellen Sequenz, die für $GABA_B$-Rezeptorproteine kodiert, enthalten.

Eine weitere erfindungsgemäße Ausgestaltung der oben beschriebenen transgenen Tiere sind transgene Tiere, in dessen Keimzellen oder der Gesamtheit oder einem Teil der somatischen Zellen; oder in dessen Keimzellen und der Gesamtheit oder einem Teil der somatischen Zellen die erfindungsgemäßen Nukleotidsequenz durch gentechnische Verfahren verändert oder durch Einfügen von DNA-Elementen unterbrochen wurden.

Die Kombination aus dem Wirtsorganismen und den zu den Organismen passenden Vektoren wie Plasmide, Viren oder Phagen wie beispielsweise Plasmide mit dem RNA-Polymerase/Promoter System, die Phagen 1, Mu oder andere temperänte Phagen oder Transposons und/oder weiteren vorteilhaften regulatorischen Sequenzen bilden ein Expressionssystem. Bevorzugt sind unter dem Begriff Expressionssysteme beispielsweise die Kombination aus Säugetierzellen wie CHO-Zellen und vektoren wie pcDNA3neo-Vektor oder HEK293-Zellen und CMV-Vektor, die für Säugetierzellen geeignet sind, zu verstehen.

Die In-situ Hybridisierung mit der Sequenz SEQ ID NO: 2 oder Teilen davon ergab eine starke Expression in Hippokampus, Cortex, Cerebellum sowie in thalamischen Kernen (siehe **Fig.** 1 und Beispiele). **Fig.** 1a und 1b geben die Expressionsanalyse der zu SEQ ID NO: 1 korrespondierenden mRNA wieder. 1a zeigt den Northern blot, 1b die in situ-Hybridisierung (siehe Beispiel 3 und 4). Das Expressionsmuster überlappt mit dem des GABAB-Rezeptors und deutet auf eine wichtige zentralnervöse Funktion des in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Proteins hin. Der Hippokampus ist die entscheidende Hirnstruktur für die Speicherung neuer Gedächtnisinhalte. Ein Protein mit der Sequenz SEQ ID NO: 2 ist damit ein interessantes Target für das Verständnis in Bezug auf Lernen und Gedächtnis und für die Entwicklung neuer kognitiver Enhancer. Als Teil des limbischen Systems beeinflußt der Hippocampus auch Stimmungen und Gefühle. Gegen SEQ ID NO: 2 oder SEQ ID NO: 4 und ihre funktionellen Äquivalente, Homologe oder Derivate gerichtete Pharmaka stellen damit potentielle Antidepressiva oder Anxiolytika dar und können bei cognitiven Erkrankungen verwendet werden. Schließlich ist der Hippokampus stark in Temporallappenepilepsien involviert, was ein Protein mit SEQ ID NO: 2 oder SEQ ID NO: 4 zu einem attraktiven Target für neue Medikamente gegen diese häufige Erkrankung macht. Im Cortex befinden sich Regionen, die sensorische Informationen integrieren, verarbeiten und in geeignete

Reaktionen umwandeln. Diese sensorischen und motorischen Zentren sind oft auch Ausgangspunkte für epileptische Anfälle. Eine gezielte Beeinflussung der erfindungsgemäßen Proteine oder des erfindungsgemäßen Proteinheteromer könnte die Krampfwahrscheinlichkeit bei Epilepsipatienten senken. Die thalamischen Kerne sind dem Cortex vorgeschaltet, integrieren die über die Sinnesorgane aufgenommenen Wahrnehmungen und leiten sie an corticale Strukturen weiter. Sie sind häufig der Ausgangspunkt für generalisierte Krampfanfälle. Die starke Expression der erfindungsgemäßen Proteine oder des Proteinheteromers in den thalamischen Kernen deutet darauf hin, daß seine Aktivierung oder seine Inhibition zur Linderung von Krampfanfällen in Epilepsipatienten beitragen kann. Die cerebellären Verknüpfungen sind maßgeblich für die Feinkoordination der Bewegungen verantwortlich. Ataxien und andere motorische Erkrankungen könnten auf einer Deregulation eines Proteins mit der erfindungsgemäßen Nukleinsäuresequenz beruhen. Die erfindungsgemäßen Proteinheteromere oder Proteine stellen damit interessante Targets für die Entwicklung neuer Substanzen dar, die zur Herstellung von Medikamenten zur Behandlung von Krankheiten wie neurologische Erkrankungen wie Epilepsie, Schlaganfall, psychische Erkrankungen wie Angst, manisch-depressive Erkrankungen, Migräne, kognitive Verluste und weiteren neurologischen Erkrankungen.

Substanzen die eine Wirkung gegenüber $GABA_B$-Rezeptoren aufweisen sind beispielsweise als Agonist Baclofen und seine Derivate und als Antagonist Phaclofen, Saclofen und Derivate. Diese Substanzen sowie weitere wirksame Substanzen sind J. Med. Chem., 38, 1995: 3313–3331, J. Med. Chem., 38, 1995: 3297–3312 und WO97/46675 zu entnehmen. Diese Substanzen wirken mit hoher Wahrscheinlichkeit auch gegenüber dem erfindungsgemäßen Proteinheteromer oder dem erfindungsgemäßen Protein. Vermutlich lassen sich mit Hilfe der erfindungsgemäßen Proteine (= Proteinheteromer + isoliertes Protein) leichter agonistisch stärker wirkende Substanzen entwickeln. Mit Hilfe der neuen Proteine können selektivere Substanzen entwickelt werden.

Das Gen für den bereits bekannten $GABA_B$-Rezeptor befindet sich in der Nähe des chromosomalen Locus, der mit der juvenilen, myoclonischen Epilepsie assoziiert ist. Diese Korrelation könnte außerdem ein neues Diagnoseverfahren für diese verbreitete Epilepsieform ermöglichen. Gleiches gilt für die erfindungsgemäßen Proteine. Die Nukleotidsequenzen SEQ ID NO: 1 und SEQ ID NO: 3 kann dazu verwendet werden, Gene für mENAs, die für diese Nukleinsäuren oder deren funktionellen Äquivalente, Homologe oder Derivate kodieren, im murinen und im menschlichen Genom mit gängigen Methoden durch Homologiescreening zu isolieren und zu kartieren und mit Markern für humane Erbkrankheiten zu korrelieren. Dies ermöglicht die Identifizierung des Gens als Ursache für bestimmte Erbkrankheiten, was ihre Diagnose erheblich vereinfacht und neue Therapieansätze ermöglicht. Mit Hilfe der Nukleinsäuren als Marker lassen sich damit Erbkrankheiten diagnostizieren.

Die Erfindung betrifft außerdem die Verwendung der erfindungsgemäßen Nukleinsäuren oder Teilen davon zur Gentherapie. Auch zu den erfindungsgemäßen Nukleinsäure oder Teilen davon komplementäre Sequenzen können zur Gentherapie verwendet werden.

Eine weitere Möglichkeit des Einsatzes der Nukleotidsequenz oder Teilen davon ist die Erzeugung transgener oder knock-out- oder konditioneller oder regionenspezifischer knock-out Tiere oder spezifischer Mutationen in gentechnisch veränderten Tieren (Ausubel et al. [eds]. 1998, Current Protocols in Molecular Biology, John Wiley & Sons, New York und Torres et al., [eds.]in 1997, Laboratory protocols for conditional gene targeting, Oxford University Press, Oxford). Über transgene überexpression oder genetische Mutation (Nullmutation oder spezifische Deletionen, Insertionen oder Veränderungen) durch homologe Rekombination in embryonalen Stammzellen kann man Tiermodelle erzeugen, die wertvolle weitere Informationen über die (Patho-)Physiologie der erfindungsgemäßen Sequenzen allein oder in Komplex mit dem $GABA_B$-Rezeptor liefern. Solchermaßen hergestellte Tiermodelle können essentielle Testsysteme zur Evaluierung neuartiger Therapeutika darstellen, die die Signaltransduktion von $GABA_B$-Rezeptoren beeinflussen.

Die Interaktion des bekannten $GABA_B$-Rezeptors mit dem neuen erfindungsgemäßen, beschriebenen 7-Transmembranregionen-Protein, das mit dem two-hybrid System entdeckt wurde, spielt eine wichtige physiologische Rolle. Dieser überraschende Befund ermöglicht neue außerordentliche Behandlungsmöglichkeiten im Hinblick auf die o. g. neurologischen und psychischen Erkrankungen, die mit dem $GABA_B$-Rezeptor im Zusammenhang stehen. Niedermolekulare Effektoren oder Peptide, die diese Interaktion positiv oder negativ beeinflussen, sind Wirkstoffe, die in die GABAerge Signaltransduktion eingreifen und damit als neue Klasse von Pharmaka zum Einsatz kommen können. Die direkte molekulare Interaktion zwischen zwei unterschiedlichen metabotropen Rezeptoren war bislang nicht bekannt. Ebenso sind bislang keine Substanzen beschrieben, die die Interaktion zwischen zwei unterschiedlichen metabotropen Rezeptoren beeinflussen und dadurch die Signaltransduktion über diese Rezeptoren modulieren. Die Verwendung des erfindungsgemäßen Proteinheteromer ermöglicht damit die Entwicklung neuer Wirkstoffe bzw. Wirkstoffklassen.

Durch Verwendung der erfindungsgemäßen Nukleinsäuresequenz, des Nukleinsäurekonstrukts, eines erfindungsgemäßen Proteinheteromeren oder des Proteins können Proteine identifiziert werden, die zum Proteinheteromeren oder zum Protein spezifische Bindungsaffinitäten aufweisen, oder zur Identifizierung von Nukleinsäuren, die für Proteine kodieren, die zum Proteinheteromeren oder zum Protein spezifische Bindungsaffinitäten aufweisen. Vorteilhafterweise werden hierzu das two-hybrid System oder andere biochemische Verfahren allein oder in Kombination verwendet. Es lassen sich so Interaktionsdomänen von metabotropen Rezeptoren und damit pharmakotherapeutische Interventionspunkte bestimmen.

Daher ist ein Gegenstand der Erfindung die Verwendung des two-hybrid Systems oder biochemischer Verfahren zur Identifizierung der Interaktionsdomänen von metabotropen Rezeptoren und die Verwendung zur pharmakotherapeutischen Intervention.

Über Strukturanalysen des Proteinheteromer oder des erfindungsgemäßen Proteins lassen sich gezielt Substanzen finden, die eine spezifische Bindungsaffinität aufweisen.

Die beschriebenen Sequenzen SEQ ID NO: 1 und SEQ ID NO: 3 ermöglichen, mit Hilfe des two-hybrid Systems oder anderen Assays, die für die Interaktion verantwortlichen Aminosäuren einzugrenzen und Substanzen zu finden, mit denen die Interaktion zwischen den beiden metabotropen Rezeptoren beeinflusst werden kann. Die Verwendung von Substanzen, die die physikalische Interaktion von zwei metabotropen Rezeptoren beeinflussen, für die Behandlung von Krankheiten, stellt unabhängig von dem speziellen hier für den $GABA_B$-Rezeptor beschriebenen Fall ein neues pharma-

kologisches Prinzip dar.

Dies ermöglicht ein Verfahren zum Auffinden von Substanzen mit spezifischer Bindungsaffinität zum erfindungsgemäßen Proteinheteromer bzw. Protein, das folgende Schritte umfaßt.

a) Inkubation des oder der Proteine mit der zu testenden Substanz.
b) Detektion der Bindung der zu testenden Substanz an das Protein.

Die Detektion der Bindung erfolgt durch Messen der Antagonisierung oder Agonisierung der $GABA_B$-Rezeptor-Aktivität oder durch Messen einer physiologischen Wirkung, wie z. B. einer Änderung der Calcium-, cAMP-, IP3-Konzentration oder des Membranpotentials.

Weitere Ausgestaltungsformen der Erfingung sind ein Verfahren zum Auffinden von Substanzen, die die Interaktion von Proteinen mit Aminosäuresequenzen, wie sie in SEQ ID NO: 2 bzw. SEQ ID NO: 4 dargestellt werden, mit anderen metabotropen Rezeptoren hemmen oder verstärken; ein Verfahren zum Auffinden von Substanzen, die die Interaktion von Liganden mit dem erfindungsgemäßen Proteinheteromer oder dem erfindungsgemäßen Proteinen mit Aminosäuresequenzen wie SEQ ID NO: 2 bzw. SEQ ID NO: 4 hemmen oder verstärken oder ein Verfahren zum Auffinden von Substanzen, die die Interaktion von Proteinen mit Aminosäuresequenzen wie SEQ ID NO: 2 bzw. SEQ ID NO: 4 mit G-Proteinen oder anderen Signaltransduktionsmolekülen hemmen oder verstärken. Die Interaktion von Proteinen mit den erfindungsgemäßen Aminosäuren läßt sich mit Hilfe des Two-hybrid Systems detektieren. Weiter lassen sich die Verfahren durch Expression der Proteine in eukaryotischen Zellen und Verknüpfung mit einem Reporter-Assay für die Aktivierung des $GABA_B$-Rezeptors durchführen. Dabei wird beispielsweise die Änderung des cAMP-Spiegels oder des Membranpotentials detektiert.

Weiterhin betrifft die Erfindung ein Verfahren zur qualitativen und quantitativen Bestimmung von Proteinen mit Aminosäuresequenzen wie SEQ ID NO: 2 oder SEQ ID NO: 4 unter Benutzung von spezifischen Agonisten oder Antagonisten. Dabei wird die GABAB-Rezeptor-Ligandenbindung zur Detektion ausgenutzt.

Über Antikörper kann die Proteinaktivität der Proteine mit den Sequenzen SEQ ID NO: 2 oder SEQ ID NO: 4 bestimmt werden. Daher ist ein weiterer Gegenstand der Erfindung ein Verfahren zur Quantifizierung der Proteinaktivität eines Proteins mit den Sequenzen SEQ ID NO: 2 oder SEQ ID NO: 4.

Die regulatorischen Sequenzen der erfindungsgemäßen Nukleinsäuren, insbesondere der Promotor, die Enhancer, locus control regions und silencer oder jeweilige Teilsequenzen davon können für die gewebespezifische Expression von diesem und weiteren Genen verwendet werden. Damit ergibt sich die Möglichkeit, gehirnspezifische Genexpression von Nukleinsäurekonstrukten durchzuführen.

Um ein DNA-Fragment zu isolieren, das die Bereiche enthält, die die Transkription der Sequenzen SEQ ID NO: 1 oder SEQ ID NO: 3 regulieren, wird zunächst eine genomische Bank mit einer möglichst weit 5'-positionierten cDNA-Probe durchsucht. Dazu wird eine dem Fachmann geläufige Homologiesuche durchgeführt (Ausubel et al. [eds.], 1998, Current Protocols in Molecular Biology, John Wiley & Sons, New York). Auf dem isolierten DNA-Fragment wird dann der Transkriptionsstart identifiziert. Der Bereich vor dem Transkriptionsstart wird anschließend mit einem Reportergen wie β-Galactosidase oder GFP (= green fluorescent protein) verknüpft und in Zellen oder in transgenen Tieren wie Mäusen daraufhin getestet, ob er zu dem für SEQ ID NO: 1 oder SEQ ID NO: 3 spezifischen Expressionsmuster führt (Ausubel et al. siehe oben). Das Reportergen kann anschließend mit anderen cDNAs verknüpft werden, um Tiermodelle zu erstellen, in denen die jeweilige cDNA regionspezifisch exprimiert wird (siehe beispielsweise Oberdick et al., Science, 248, 1990: 223–226).

Ausgehend von den Aminosäuresequenzen SEQ ID NO: 2 oder SEQ ID NO: 4 können synthetische Peptide generiert werden, die als Antigene für die Produktion von Antikörpern eingesetzt werden. Es ist auch möglich, das Polypeptid oder Bruchstücke davon zur Generierung von Antikörpern einzusetzen. Mit Antikörpern sind sowohl polyklonale, monoklonale, humane oder humanisierte oder rekombinante Antikörper oder Fragmente davon, single chain Antikörper oder auch synthetische Antikörper gemeint. Unter erfindungsgemäßen Antikörpern oder deren Fragmente sind prinzipiell alle Immunoglobulinklassen wie IgM, IgG, IgD, IgE, IgA oder ihre Subklassen wie die Subklassen des IgG oder deren Mischungen zu verstehen. Bevorzugt sind IgG und seine Subklassen wie beispielsweise IgG_1, IgG_2, IgG_{2a}, IgG_{2b}, IgG_3 oder IgG_M. Besonders bevorzugt sind die IgG Subtypen IgG_1/κ oder IgG_{2b}/κ. Als Fragmente seien alle verkürzten oder veränderten Antikörperfragmente mit einer oder zwei dem Antigen-komplementären Bindungsstellen, wie Antikörperteile mit einer den Antikörper entsprechenden von leichter und schwerer Kette gebildeten Bindungsstelle wie Fv-, Fab- oder $F(ab')_2$-Fragmente oder Einzelstrangfragmente, genannt. Bevorzugt sind verkürzte Doppelstrangfragmente wie Fv-, Fab- oder $F(ab')_2$. Diese Fragmente können beispielsweise auf enzymatischem Wege durch Abspaltung des Fc-Teils der Antikörper mit Enzymen wie Papain oder Pepsin, durch chemische Oxidation oder durch gentechnische Manipulation der Antikörpergene erhalten werden. Auch genmanipulierte nichtverkürzte Fragmente können vorteilhaft verwendet werden.

Die Antikörper oder Fragmente können allein oder in Mischungen verwendet werden.

Die Antikörpergene für die gentechnischen Manipulationen lassen sich in dem Fachmann bekannterweise beispielsweise aus den Hybridomzellen isolieren (Harlow, E. and Lane, D. 1988, Antibodies: A Laboratory Manual, Cold Spring Harbor Press, N. Y.; Ausubel et al., [eds], 1998, Current Protocols in Molecular Biology, John Wiley & Sons, New York). Dazu werden Antikörper-produzierende Zellen angezogen und die mRNA bei ausreichender optischer Dichte der Zellen über Zellyse mit Guanidiniumthiocyanat, Ansäuern mit Natriumacetat, Extraktion mit Phenol, Chloroform/Isoamylalkohol, Fällungen mit isopropanol und Waschen mit Ethanol aus den Zellen in bekannter Weise isoliert. Anschließend wird mit Hilfe der Reversen Transcriptase cDNA aus der mRNA synthetisiert. Die synthetisierte cDNA kann direkt oder nach genetischer Manipulation beispielsweise durch "site directed mutagenesis", Einführung von Insertionen, Inversionen, Deletionen oder Basenaustausche in geeignete tierische, pilzliche, bakterielle oder virale Vektoren inseriert und in den entsprechenden Wirtsorganismen exprimiert werden. Bevorzugt werden bakterielle oder Hefe Vektoren wie pBR322, pUC18/19, pACYC184, Lambda oder Hefe-mu-Vektoren zur Klonierung der Gene und die Expression in Bakterien wie

E. coli bzw. in der Hefe wie Saccharomyces cerevisiae.

Spezifische Antikörper gegen die erfindungsgemäßen Proteine können sich sowohl als diagnostische Reagenzien als auch als Therapeutika bei neurologischen oder psychiatrischen Krankheitsbildern eignen.

Weiterhin können die cDNA, die genomische DNA, die regulatorischen Elemente der findungsgemäßen Nukleinsäuresequenzen, als auch das Polypeptid, sowie Teilfragmente davon in rekombinanter oder nichtrekombinanter Form zur Ausarbeitung eines Testsystems verwendet werden. Dieses Testsystem ist geeignet, die Aktivität des Promotors oder des Proteins in Anwesenheit der Testsubstanz zu messen. Bevorzugt handelt es sich hierbei um einfache Meßmethoden (kolorimetrische, luminometrische, auf Fluoreszenz beruhende oder radioaktive), die die schnelle Messung einer Vielzahl von Testsubstanzen erlauben (Böhm, Klebe, Kubinyi, 1996, Wirkstoffdesign, Spektrum-Verlag, Heidelberg). Die beschriebenen Testsysteme erlauben das Durchsuchen von chemischen Bibliotheken nach Substanzen, die agonistische oder antagonistische Wirkungen auf SEQ ID NO: 2 oder SEQ ID NO: 4 oder den neuen $GABA_B$-Rezeptor-Komplex, bestehend aus dem bereits beschriebenen $GABA_B$-Rezeptor und dem in SEQ ID NO: 2 oder SEQ ID NO: 4 beschriebenen Protein, haben. Die Identifizierung solcher Substanzen stellt den ersten Schritt auf dem Weg zur Identifizierung neuartiger Medikamente dar, die spezifisch auf die GABAerge Signaltransduktion wirken.

Ein alternativer Weg zur Entwicklung von Wirkstoffen, die am neuen $GABA_B$-Rezeptor angreifen, besteht im rationalen Drug Design (Böhm, Klebe, Kubinyi, 1996, Wirkstoffdesign, Spektrum-Verlag, Heidelberg). Hier wird die Struktur oder eine Teilstruktur von dem in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Protein, soweit sie vorliegt, oder ein von Computern erstelltes Modell der Struktur, benutzt, um mit Unterstützung von Molecular Modelling Programmen Strukturen zu finden, für die sich eine hohe Affinität an den $GABA_B$-Rezeptor vorhersagen läßt. Diese Substanzen werden dann synthetisiert und getestet. Hochaffine, selektive Substanzen werden auf ihre Verwendung als Medikamente gegen Epilepsie, Schlaganfall und andere neurologische Erkrankungen getestet.

Die Bestimmung von Menge, Aktivität und Verteilung des neuen $GABA_B$-Rezeptor-Komplexes oder von dem in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Protein oder seiner zugrundeliegenden mRNA im menschlichen Körper kann zur Diagnose, Erfassung der Prädisposition und zum Monitoring bei bestimmten Erkrankungen dienen. Desgleichen kann die Sequenz der cDNA der Sequenzen SEQ ID NO: 2 oder SEQ ID NO: 4 sowie der genomischen DNA zu Aussagen über genetische Ursachen und Prädispositionen bestimmter Erkrankungen herangezogen werden. Dazu können sowohl DNA/RNA-Proben als auch Antikörper verschiedenster Art benutzt werden. Dabei dient die beschriebene Nukleotidsequenz SEQ ID NO: 1 oder SEQ ID NO: 3 oder Teile davon in Form geeigneter Proben zur Aufdeckung von Punktmutationen oder Deletionen/Insertionen/Rearrangements.

Die vorliegende Nukleinsäuresequenz SEQ ID NO: 1 oder SEQ ID NO: 3, ihre funktionellen Äquivalente, Homologe oder Derivate, das von ihr kodierte Protein (SEQ ID NO: 2 oder SEQ ID NO: 4) oder das erfindungsgemäße Proteinheteromer sowie davon abgeleitete Reagenzien (Oligonukleotide, Antikörper, Peptide) können zur Diagnose und Therapie von neurologischen Erkrankungen eingesetzt werden. Außerdem wird die Diagnose und Behandlung von genetischen Prädispositionen für bestimmte neurologische Erkrankungen wie Epilepsie, Schlaganfall, psychische Erkrankungen wie Angst, manisch-depressive Erkrankungen, Migräne, kognitive Verluste und weitere neurologische Erkrankungen möglich. Weiterhin kann ein Monitoring der Behandlung oben angegebener Erkrankungen durchgeführt werden.

Ein weiterer Gegenstand der Erfindung ist ein Verfahren zum qualitativen und quantitativen Nachweis einer erfindungsgemäßen Nukleinsäure in einer biologischen Probe, das folgende Schritte umfaßt:

a) Inkubation einer biologischen Probe mit einer bekannten Menge an erfindungsgemäßer Nukleinsäure oder einer bekannten Menge an Oligonukleotiden, die als Primer für eine Amplifikation der erfindungsgemäßen Nukleinsäure geeignet sind,
b) Nachweis der erfindungsgemäßen Nukleinsäure durch spezifische Hybridisierung oder PCR-Amplifikation,
c) Vergleich der Menge an hybridisierender Nukleinsäure oder an durch PCR Amplifikation gewonnener Nukleinsäure mit einem Mengenstandard.

Außerdem betrifft die Erfindung ein Verfahren zum qualitativen und quantitativen Nachweis eines erfindungsgemäßen Proteinheteromers oder eines erfindungsgemäßen Proteins in einer biologischen Probe, das folgende Schritte umfaßt:

a) Inkubation einer biologischen Probe mit einem Antikörper, der spezifisch gegen das Proteinheteromer oder gegen das erfindungsgemäße Protein gerichtet ist,
b) Nachweis des Antikörper/Antigenkomplexes,
c) Vergleich der Mengen des Antikörper/Antigenkomplexes mit einem Mengenstandard.

Als Standard wird üblicherweise eine biologische Probe aus einem gesunden Organismus entnommen.

Weiterhin betrifft die Erfindung ein Verfahren zum Auffinden von Substanzen, die spezifisch an ein Protein mit einer Aminosäuresequenz SEQ ID NO: 2 oder SEQ ID NO: 4 binden, das einen oder mehrere der folgenden Schritte umfaßt:

a) Expression des Proteins in eukaryotischen oder prokaryotischen Zellen.
b) Inkubation des Proteins mit den zu testenden Substanzen.
c) Nachweis der Bindung einer Substanz an den Rezeptor bzw. eines Effektes auf die Rezeptorfunktion.

Außerdem betrifft die Erfindung ein Verfahren zum Auffinden von Substanzen, die spezifisch an ein Protein mit einer Aminosäuresequenz gemäß SEQ ID NO: 2 oder SEQ ID NO: 4 bzw. an eine Nukleinsäuresequenz gemäß SEQ ID NO: 1 oder SEQ ID NO: 3 binden und dadurch hemmende oder aktivierende funktionelle Effekte auf die GABAerge Signalweiterleitung in zentralnervösen Neuronen hervorrufen.

Je nach dem Neurotransmittersystem (z. B. GABA oder Glutamat), in das der $GABA_B$-Rezeptor involviert ist, kann eine erhöhte oder eine erniedrigte $GABA_B$-Rezeptor-Aktivität zu einem Ungleichgewicht zwischen den Neurotransmit-

tersystemen und oftmals zu einer neuronalen Übererregung führen, die eine Vielzahl neurologischer Erkrankungen charakterisiert, wie z. B. Epilepsie, Schlaganfall und deren Folgen und andere.

Mangelnde neuronale Aktivität, die z. B. Demenzen charakterisiert, kann die Folge sein, wenn präsynaptische $GABA_B$ Rezeptoren an glutamatergen Synapsen überaktiviert sind und die Transmitterausschüttung so stark hemmen, daß keine Reizweiterleitung über die Synapse mehr möglich ist.

In Situationen, in denen ein Mangel an der Aktivität des erfindungsgemäßen Proteins oder des mit SEQ ID NO: 2 oder SEQ ID NO: 4 komplettierten $GABA_B$-Rezeptors herrscht, können mehrere Methoden zur Substituierung eingesetzt werden. Zum einen kann das Protein, natürlich oder rekombinant direkt, oder durch geeignete Maßnahmen in Form seiner kodierenden Nukleinsäure (d. h. DNA oder RNA) appliziert werden. Dazu können sowohl virale, als auch nichtvirale Vehikel zum Einsatz kommen. Ein weiterer Weg bietet sich durch die Stimulation des endogenen, körpereigenen Genes durch geeignete Substanzen. Solche Substanzen lassen sich beispielsweise auffinden, indem man ihre Wirkung auf die Transkriptionselemente des neuen $GABA_B$-Rezeptor-Genes ermittelt.

In Situationen, in denen ein Überschuß an Aktivität des ein Protein mit der Sequenz SEQ ID NO: 2 oder SEQ ID NO: 4 beinhaltenden $GABA_B$-Rezeptors oder von einem Protein mit SEQ ID NO: 2 oder SEQ ID NO: 4 allein herrscht, können spezifische, synthetische oder natürliche, kompetitive und nicht-kompetitive Antagonisten gegen das Protein mit der Sequenz SEQ ID NO: 2 oder SEQ ID NO: 4 oder Antikörper oder Antikörperfragmente gegen das Protein mit der Sequenz SEQ ID NO: 2 oder SEQ ID NO: 4 oder gegen das Proteinheteromer eingesetzt werden. Desweiteren kann sowohl durch antisense Moleküle oder Ribozyme oder Oligonukleotide als auch durch niedermolekulare Verbindungen eine Inhibition der $GABA_B$-Rezeptor-Aktivität bzw. der Aktivität des Proteins mit der Sequenz SEQ ID NO: 2 oder SEQ ID NO: 4 erreicht werden.

Beispiele

Die Nukleotidsequenz mit der in SEQ ID NO: 1 dargestellten Struktur wurde aus einer cDNA Bibliothek aus Ratten-Gehirn identifiziert. Bei der Suche nach Proteinen, die mit dem intrazellulären Carboxyterminus des bekannten $GABA_B$-Rezeptors interagieren, wurde die Nukleotidsequenz gefunden. Im Experiment wurde eine cDNA-Bibliothek aus Ratten-Gehirn mit dem two-hybrid System nach Interaktionspartnern des Carboxyterminus des oben beschriebenen $GABA_B$-Rezeptors durchsucht. Mehrere überlappende Fragmente einer unbekannten cDNA wurden gefunden. Mit Hilfe dieser Fragmente wurde aus einer cDNA-Bibliothek aus Ratten-Hippokampus ein 5kb langes Fragment der unbekannten cDNA durch Homologiescreening isoliert und anschließend sequenziert. Die so gewonnene cDNA-Sequenz enthält den kompletten kodierenden Bereich für die Sequenz SEQ ID NO: 2. Die Entdeckung und molekulare Charakterisierung von Interaktionspartnern des bekannten klonierten $GABA_B$-Rezeptors ermöglicht es, die physiologischen Eigenschaften und die biochemische und pharmakologische Vielfalt des $GABA_B$-Rezeptors besser zu verstehen, sowie neue konkrete Angriffspunkte für pharmakotherapeutische Interventionen zu erhalten.

Die Sequenzanalyse des durch die vorliegende cDNA (= SEQ ID NO: 1) kodierten Polypeptides läßt darauf schließen, daß es sich um einen metabotropen Rezeptor handelt. Es enthält eine aminoterminale Signalsequenz [Aminosäuren 1–40 (G. von Heijne, N. A. R., 14, 4683, 1986)] sowie sieben charakteristisch angeordnete hydrophobe Bereiche, die mit hoher Wahrscheinlichkeit die Plasmamembran durchspannen. Das Vorhandensein dieser sieben hydrophoben Bereiche ist ein Charakteristikum von metabotropen Rezeptoren. Sequenzvergleiche (Altschul et al., J. Mol. Biol., 215, 403–410, 1990) der Aminosäuresequenz SEQ ID NO: 2 mit den Sequenzen der offenen Leseraster der translatierten öffentlichen Nukleotiddatenbanken (nrdb) mit Hilfe des BLAST-Programmes (Version BLASTP 2.0a19-WashU [05-Feb-1998]) zeigten Ähnlichkeiten zu dem oben beschriebenen $GABA_B$-Rezeptor (höchste Bewertung (best score): 36% Sequenzidentität über 804 Aminosäuren mit $GABA_B$-Rezeptor 1B und 36% Sequenzidentität über 744 Aminosäuren mit $GABA_B$-Rezeptor 1A) sowie eine wesentlich geringere Sequenzähnlichkeit zu metabotropen Glutamatrezeptoren und Calciumsensitiven Rezeptoren. Die Ähnlichkeit erstreckt sich über den aminoterminalen Bereich sowie besonders über die Transmembranregionen, aber nicht über den carboxyterminalen, intrazellulären Bereich (siehe **Fig.** 2). Bei dem in SEQ ID NO: 2 beschriebenen Protein handelt es sich demnach um einen neuen metabotropen Rezeptor, der entweder allein oder in Komplex mit dem bekannten $GABA_B$-Rezeptor Signalweiterleitung im ZNS vermittelt oder diese reguliert.

Fig. 2 gibt einen Sequenzvergleich des erfindungsgemäßen Proteins mit der SEQ ID NO: 2 mit den bekannten GABAB-Rezeptor-Proteinen (= GBR) 1A und 1B (Kaupmann et al. 1997, siehe oben) wieder. Identische Aminosäuren sind durch schwarze, konservative Veränderungen durch hellgraue Schattierungen dargestellt. Die sieben Transmembranregionen (= TM1 bis TM7) sind durch darüberliegende Balken markiert.

Die Analyse der Verteilung der mRNA, von der die cDNA-Sequenz SEQ ID NO: 1 stammte, wurde mittels Northern Blot und mittels In-situ Hybridisierung auf Rattenhirnschnitten durchgeführt. Eine Analyse in 10 verschiedenen Ratten-Geweben ergab eine gehirnspezifische Expression einer 5–6 kb großen mRNA. In sehr geringem Umfang läßt sich eine kleinere mRNA in Ratten-Testis nachweisen (siehe **Fig.** 1). Die In-situ Hybridisierung ergab eine starke Expression in Hippokampus, Cortex, Cerebellum sowie in thalamischen Kernen. Das Expressionsmuster überlappt mit dem des $GABA_B$-Rezeptors und deutet auf eine wichtige zentralnervöse Funktion des in SEQ ID NO: 2 dagestellten Proteins hin.

Soweit nicht anders angegeben wurde bei der experimentellen Durchführung entsprechend den Vorschriften in "Ausubel et al. (eds.), 1998. Current Protocols in Molecular Biology. John Wiley& Sons, New York" verfahren.

Beispiel 1

Two-hybrid Suche mit dem Carboxyterminus von $GABA_B$-Rezeptor 1

Die für den Carboxyterminus des $GABA_B$-Rezeptor 1A kodierende cDNA (Aminosäuren 857–960, accession no. Y10369, EMBL-Datenbank) wurde mit den spezifischen Primern GABA-CT5' (5'-GCGAATTCCGCAGGCTGATCACCCGAGGG-3') und GABA-CT3' (5'-GCAGTCGACTCACTTGTAAAGCAAATGTACTCG-3') von Rattenhirn

cDNA in einer Polymerase-Ketten-Reaktion (PCR) amplifiziert, mit den Enzymen EcoRI und SalI einer Restriktion unterzogen und danach über die überhängenden Enden in einen mit den Enzymen EcoRI und SalI vorgeschnittenenen Vektor pGBT (Firma Clontech) kloniert. Das so entstandene DNA-Konstrukt(pGBT-GABA$_B$-Rezeptor 1) kodiert für ein Protein, in dem die Gal4-DNA-Bindungsdomäne mit dem C-terminus des GABA$_B$-Rezeptors fusioniert ist. Mit diesem Konstrukt wurde der Hefestamm HF7c (Firma Clontech) transformiert. Der resultierende Hefestamm wurde mit einer Rattenhirn cDNA Bank (Kornau et al., Science 269, 1737–1740, 1995) im Vektor pGAD (Firma Clontech) transformiert und 4×10^6 Transformanden auf Tryptophan/Leucin/Histidin-Mangelmedium plattiert. Nach 3, 4 und 5 Tagen Wachstum bei 30°C wurden Kolonien mit einem Durchmesser von mehr als 2 mm vereinzelt und einer XGal-Färbung unterzogen. Insgesamt 7 Kolonien erwiesen sich als His3 und lacZ positiv (pGAD-pos1–7). Aus diesen wurde die jeweilige cDNA aus dem Vektor pGAD mit dem vektorspezifischen Primer Gal4AD3' (5'-AAGAGATCCTAGAACTAGTGGATC-3') sowie T7 (5'-CGTAATACGACTCACTATAGGGCG-3') amplifiziert und das Amplikon sequenziert. Die Sequenzanalyse ergab 5 verschiedene überlappende Fragmente (Nukleotidsequenzen 2399 3102 (a) 2432 3102 (b), 2447 3102 (c), 2462–3102 (d), 2468–3102 (e) in der Sequenz SEQ ID NO: 1).

Aus zwei verschiedenen positiven Kolonien (a und e) wurde die pGAD-Plasmid-DNA aufgereinigt und mit verschiedenen pGBT-Konstrukten in den Hefestamm HF7c kotransformiert. Nur in Kombination mit dem Konstrukt pBGT-GABA$_B$-Rezeptor 1 konnte eine Aktivierung der Reportergene His3 und lacZ festgestellt werden.

Beispiel 2

Klonierung der cDNA für die neue GABA$_B$-Rezeptor-Komponente SEQ ID NO: 2

Ein aus der two-hybrid Suche gewonnenes cDNA-Fragment gemäß Beispiel 1 (a, Nukleotide 2399–3102 in Sequenz SEQ ID NO: 1) wurde mit dem random primed labelling kit (Boehringer Mannheim) entsprechend den Herstellerangaben mit α-^{32}P-dCTP radioaktiv markiert. Die durch Erhitzen denaturierte radioaktive Sonde wurde auf 18 Nitrocellulose-Filter, auf die je 40000 Plaques einer cDNA-Bank aus Ratten-Hippokampus im Bakteriophagen λ transferiert worden waren, 16 Stunden lang hybridisiert (42°C, 5 × SSC, 50% Formamid) und daraufhin mehrfach mit 0,2 × SSC, 60°C gewaschen. Von 30 positiven λ-Klonen wurden 6 vereinzelt, Phagen-DNA isoliert und kartiert. Die beiden längsten cDNA-Fragmente (5kb) wurden vollständig sequenziert. Sie enthalten ein offenes Leseraster für die Aminosäuresequenz SEQ ID NO: 2. Die Sequenzanalyse ergab bei diesen beiden Lambda-cDNA-Klonen vier Unterschiede in der kodierenden Sequenz, von denen 3 stille Mutationen darstellen (Nukleotid 696 C zu T, Nukleotid 1104 T zu C, Nukleotid 2295 C zu T) und eine für ein zusätzliches Prolin in der aminoterminalen Signalsequenz kodiert (Insertion von CCG bei Nukleotid 171/172).

Beispiel 3

Expression der mRNA für die neue GABA$_B$-Rezeptor-Komponente in Ratten-Geweben

Ein aus der two-hybrid Suche gewonnenes cDNA-Fragment gemäß Beispiel 1 (a, Nukleotide 2399 3102 in SEQ ID NO: 1) wurde mit dem random primed labelling kit (Boehringer Mannheim) entsprechend den Herstellerangaben mit α-^{32}P-dCTP radioaktiv markiert. Die durch Erhitzen denaturierte radioaktive Sonde wurde mit einem Multiple Tissue Northern Blot (je 10 µg total RNA von Ratten-Gehirn, -Leber, -Lunge, -Herz, -Niere, -Hoden, -Muskel und -Darm, isoliert nach "Chomczinski and Sacchi, Anal. Biochem., 162, 156–159, 1987") in QuickHyb-Lösung (Firma Stratagene) für eine Stunde bei 68°C hybridisiert und daraufhin bei 60°C und 0,1 × SSC gewaschen. Nach drei Tagen Exposition wurde ein starkes Hybridisierungssignal auf Gehirn-RNA bei etwa 5–6 kb, ein wesentlich schwächeres Signal geringerer Größe auf Hoden-RNA und kein Signal in allen anderen untersuchten Geweben festgestellt (siehe **Fig.** 1a).

Beispiel 4

Expression der mRNA für die neue GABA$_B$-Rezeptor-Komponente im Ratten-Gehirn

Wenn nicht anders beschrieben, wurde die in-situ Hybridisierung durchgeführt wie in "Molecular Neurobiology: A Practical Approach. J. Chad and H. Wheat, eds. (Oxford: IRL Press), pp.205–225" beschrieben.

Zwei Antisense-Oligonukleotide (revers komplementär zu den Nukleotiden 2463–2498 bzw. Nukleotiden 2538–2573 in der Sequenz SEQ ID NO: 1) wurden mit Terminaler Desoxynukleotid-Transferase (Boehringer Mannheim) entsprechend den Herstellerangaben mit α-^{35}S-dATP radioaktiv markiert. Diese radioaktiven Sonden wurden auf etwa 15 µm dicke horizontale Rattenhirnschnitte appliziert und 16 Stunden lang in 4 × SSC, 50% Formamid bei 42°C hybridisiert. Daraufhin wurden die Schnitte für 30 Minuten in 1 × SSC, 55°C gewaschen und für 8 Tage exponiert. Die Autoradiogramme für beide Oligonukleotide zeigten ein übereinstimmendes Bild (siehe **Fig.** 1b). Das stärkste Signal wurde in Purkinje-Zellen des Kleinhirns, ebenfalls starke Signale in Kortex, Hippokampus und in verschiedenen thalamischen Kernen sowie schwächere Signale in den Körnerzellen des Kleinhirns gefunden.

Beispiel 5

Klonierung und Sequenzierung der cDNA für die humane Form der neuen GABA$_B$-Rezeptorkomponente SEQ ID NO: 3 (DNA-Sequenz) bzw. SEQ ID NO: 4 (Aminsosäuresequenz)

Ausgehend von 5 µg totaler RNA aus dem Gesamthirn eines 57 Jahre alten Mannes (Firma Clontech) wurde mit Superscript Reverser Transkriptase (Gibco BRL) entsprechend den Hinweisen des Herstellers cDNA synthetisiert. Mit

mehreren Oligonukleotidprimern, die von SEQ ID NO: 1 bzw. von in der EMBL-Datenbank befindlichen humanen EST-Sequenzen abgeleitet wurden, konnten in RCR-Reaktionen von der humanen cDNA spezifische Produkte amplifiziert werden. Hierbei handelte es sich um folgende Primer-Paare:

GB1s/GB6as
GB15s(hs)/GB18as
GB17s/GB11as
GB17s/GB16as(hs)
GB25s/GB4as
GB25s/GB23as(hs)
GB25sXbaI/GB23as(hs)
GB22s(hs)/GB16as(hs)

GB1s: 5'-CAGATCCGCAACGAGTCACTCCTG-3'
GB2as: 5'-CAGGAGTGACTCGTTGCGGATCTG-3'
GB3s: 5'-CAGTTTGACCAGAATATGGCAGC-3'
GB4as: 5'-GCTGCCATATTCTGGTCAAACTG-3'
GB6as: 5'-GACCTTCACCTCTCTGCTGTCTTG-3'
GB11as: 5'-GAAGGAGGGTGGTACGTGTCTGTG-3'
GB15s(hs): 5'-CTACGATGGCATCTGGGTCATC-3'
GB16as(hs): 5'-GTCCCATTTCCGTTCCTCTTC-3'
GB17s: 5'-CTCAACGACAGCAAGTACATC-3'
GB18as: 5'-GATGTACTTGCTGTCGTTGAG-3'
GB19as(hs): 5'-GCTCTAGACCGTATTTTATTGCATCGTAG-3'
GB22s(hs): 5'-GCGAATTCACAAAAAGACAAGACCATCATCCTG-3'
GB23as(hs): 5'-GCGAATTCAGGATGGTGAGGGCAGAGAGGATG-3'
GB25s: 5'-GTGAATTCGCGGCGCGGCATGGCTTC-3'
GB25sXbaI: 5'-GTTCTAGACGCGGCGCGGCATGGCTTC-3'
GB27as: 5'-CTGGTCCCGGGTCAGGAAGGAGAC-3'

Die PCR-Produkte wurden direkt mit den Primern, die schon zur Amplifikation benutzt worden waren bzw. mit den oben genannten Primern, sequenziert. Aus den erhaltenen Sequenzen wurden neue Primer abgeleitet und für die PCR-Reaktionen und Sequenzierungen eingesetzt (Liste der Primer siehe oben). Aus den Einzelsequenzen der erhaltenen PCR-Produkte konnte schließlich die Sequenz SEQ ID NO: 3 zusammengesetzt werden, die das offene Leseraster für ein Protein der Aminosäuresequenz SEQ ID NO: 4 enthält.

An den Positionen 360 und 2605 in der Sequenz SEQ ID NO: 3 wurde nach wiederholtem Sequenzieren aus beiden Richtungen neben dem Aauch ein G-Signal detektiert, so daß an dieser Stelle die Sequenz entweder A oder G lautet. Diese Änderung gegenüber SEQ ID NO: 3 würde für Position 360 zu keiner Änderung der Aminosäuresequenz SEQ ID NO: 4 führen; an Position 2605 würde es zu einem Threoninzu Alanin-Austausch an Position 869 der Sequenz SEQ ID NO: 4 führen. Die Basenpaare 1 bis 8 in SEQ ID NO: 3 wurden durch den von 1 SEQ ID NO: 1 abgeleiteten Primer GB25s vorgegeben. Es kann daher nicht ausgeschlossen werden, daß eine oder mehrere Positionen in den Positionen 1 bis 8 in der SEQ ID NO: 3 anders lauten als angegeben. Demzufolge kann auch eine oder mehrere der Aminosäuren 1 bis 3 in SEQ ID NO: 4 anders lauten als angegeben.

Das PCR-Produkt GB25sXbaI/GB23as(hs) wurde einem Restriktionsverdau mit XbaI und BglII unterzogen. Das PCR-Produkt GB22s(hs)/GB16as(hs) wurde einem Restriktionsverdau mit BglII und XhoI unterzogen, Die beiden PCR-Produkte wurden in einen mit XbaI und XhoI vorgeschnittenen pBSIIKS(–)-Vektor (Firma Stratagene) kloniert (pBS-hsGB).

SEQUENZPROTOKOLL

(1) ALGEMEINE INFORMATION:

(i) ANMELDER:
(A) NAME: BASF-LYNX Bioscience AG
(B) STRASSE: Im Neuenheimer Feld 519
(C) ORT: Heidelberg
(D) BUNDESLAND: Baden-Wuerttemberg
(E) LAND: Deutschland
(F) POSTLEITZAHL: 69120

(ii) ANMELDETITEL: Neuer metabotroper GABA-Rezeptor-Komplex aus dem zentralen Nervensystem

(iii) ANZAHL DER SEQUENZEN: 4

(iv) COMPUTER-LESBARE FORM:
(A) DATENTRÄGER: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) BETRIEBSSYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPA)

(2) INFORMATION ZU SEQ ID NO: 1:

(i) SEQUENZ CHARAKTERISTIKA:
(A) LÄNGE: 3288 Basenpaare
(B) ART: Nukleinsäure
(C) STRANGFORM: Einzel
(D) TOPOLOGIE: linear

(ii) ART DES MOLEKÜLS: cDNS zu mRNS

(iii) HYPOTHETISCH: NEIN

(iii) ANTISENSE: NEIN

(vi) URSPRÜNLICHE HERKUNFT:
(A) ORGANISMUS: Rattus norvegicus

(ix) MERKMALE:
(A) NAME/SCHLÜSSEL: CDS
(B) LAGE: 118..2940

(xi) SEQUENZBESCHREIBUNG: SEQ ID NO: 1:

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CCGGGGCCCG TGCCGGCGCC ATTGCGCGGG AGCCGCGGGC AAAGCTCGGC GCCGGGCGGC      60

GGGCCGGGCC AGGCCATGCG GGCCGAGTGA GCTGGCGCCC GCAGCCCGCG GCGCGGC       117

ATG GCT TCC CCG CCG AGC TCC GGG CAG CCC CGG CCG CCG CCG CCG CCG       165
Met Ala Ser Pro Pro Ser Ser Gly Gln Pro Arg Pro Pro Pro Pro Pro
 1               5                  10                  15
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CCG CCG CCC GCG CGC CTG CTG CTG CCC CTG CTG CTG TCG CTG CTG CTG      213
Pro Pro Pro Ala Arg Leu Leu Leu Pro Leu Leu Leu Ser Leu Leu Leu
            20                  25                  30

TGG TTG GCG CCC GGG GCC TGG GGC TGG ACG CGG GGC GCC CCC CGG CCG      261
Trp Leu Ala Pro Gly Ala Trp Gly Trp Thr Arg Gly Ala Pro Arg Pro
        35                  40                  45

CCG CCC AGC AGC CCG CCG CTC TCC ATC ATG GGC CTC ATG CCG CTC ACC      309
Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu Thr
    50                  55                  60

AAG GAG GTG GCC AAG GGC AGC ATC GGG CGC GGC GTG CTC CCC GCC GTG      357
Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala Val
65                  70                  75                  80

GAG CTA GCC ATC GAG CAG ATC CGC AAC GAG TCA CTC CTG CGC CCC TAC      405
Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro Tyr
                85                  90                  95

TTC CTG GAC CTG CGA CTC TAT GAC ACC GAG TGT GAC AAT GCA AAG GGA      453
Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys Gly
            100                 105                 110

CTG AAA GCC TTC TAT GAC GCA ATA AAG TAT GGG CCG AAC CAT TTG ATG      501
Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu Met
        115                 120                 125

GTG TTT GGA GGC GTC TGT CCG TCT GTC ACA TCT ATT ATC GCG GAG TCC      549
Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu Ser
    130                 135                 140

CTC CAA GGC TGG AAT CTG GTG CAG CTT TCC TTC GCC GCC ACC ACG CCT      597
Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr Pro
145                 150                 155                 160

GTT CTT GCG GAT AAG AAG AAG TAC CCG TAT TTC TTC CGG ACG GTG CCG      645
Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val Pro
                165                 170                 175

TCA GAC AAC GCG GTG AAC CCC GCC ATC CTG AAG CTC CTG AAG CAC TTC      693
Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His Phe
            180                 185                 190

CGC TGG CGG CGT GTG GGC ACA CTC ACG CAG GAC GTG CAG CGC TTC TCC      741
Arg Trp Arg Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe Ser
        195                 200                 205

GAG GTG AGG AAT GAC CTG ACT GGG GTT CTG TAT GGG GAA GAT ATT GAG      789
Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile Glu
    210                 215                 220
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ATC TCA GAC ACA GAG AGC TTC TCC AAT GAT CCC TGC ACC AGC GTC AAA          837
Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val Lys
225                 230                 235                 240

AAG CTC AAG GGG AAT GAC GTG CGG ATC ATC CTT GGC CAG TTT GAC CAG          885
Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp Gln
                245                 250                 255

AAT ATG GCA GCA AAA GTC TTC TGT TGT GCC TTC GAG GAG AGC ATG TTT          933
Asn Met Ala Ala Lys Val Phe Cys Cys Ala Phe Glu Glu Ser Met Phe
            260                 265                 270

GGC AGC AAG TAC CAG TGG ATC ATC CCG GGA TGG TAC GAG CCT GCG TGG          981
Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ala Trp
        275                 280                 285

TGG GAG CAG GTG CAT GTG GAG GCC AAT TCC TCA CGC TGC CTG CGC AGA         1029
Trp Glu Gln Val His Val Glu Ala Asn Ser Ser Arg Cys Leu Arg Arg
    290                 295                 300

AGC CTC CTG GCT GCC ATG GAA GGT TAC ATC GGA GTG GAC TTT GAG CCC         1077
Ser Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu Pro
305                 310                 315                 320

CTG AGC TCC AAA CAA ATC AAG ACC ATT TCA GGG AAG ACT CCA CAG CAG         1125
Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln Gln
                325                 330                 335

TAT GAA AGA GAG TAC AAC AGC AAA CGT TCA GGC GTG GGG CCC AGC AAG         1173
Tyr Glu Arg Glu Tyr Asn Ser Lys Arg Ser Gly Val Gly Pro Ser Lys
            340                 345                 350

TTC CAT GGG TAC GCC TAC GAT GGG ATC TGG GTC ATC GCC AAG ACC CTA         1221
Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr Leu
        355                 360                 365

CAG AGG GCC ATG GAG ACA CTG CAT GCC AGT AGC AGG CAC CAG CGG ATC         1269
Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg Ile
    370                 375                 380

CAG GAC TTC AAC TAC ACA GAC CAC ACG CTG GGC AAA ATC ATC CTC AAT         1317
Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Lys Ile Ile Leu Asn
385                 390                 395                 400

GCC ATG AAC GAG ACC AAC TTC TTC GGG GTC ACG GGT CAA GTT GTG TTC         1365
Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val Phe
                405                 410                 415

CGG AAC GGG GAG AGA ATG GGA ACC ATT AAA TTT ACT CAA TTT CAA GAC         1413
Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln Asp
            420                 425                 430
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AGC AGA GAG GTG AAG GTC GGC GAA TAC AAC GCG GTG GCT GAC ACA CTG      1461
Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr Leu
        435                 440                 445

GAG ATC ATC AAT GAC ACC ATA AGG TTC CAG GGG TCC GAG CCA CCC AAG      1509
Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro Lys
    450                 455                 460

GAC AAG ACC ATC ATT CTG GAG CAG CTT CGG AAG ATC TCG CTT CCA CTG      1557
Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro Leu
465                 470                 475                 480

TAT AGC ATC CTG TCC GCT CTC ACC ATC CTC GGC ATG ATC ATG GCC AGC      1605
Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala Ser
                485                 490                 495

GCC TTC CTC TTC TTC AAC ATC AAG AAC CGG AAC CAA AAG CTG ATT AAG      1653
Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile Lys
            500                 505                 510

ATG TCA AGC CCC TAC ATG AAC AAC CTC ATC ATC CTG GGA GGA ATG CTG      1701
Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met Leu
        515                 520                 525

TCC TAT GCA TCC ATC TTC CTC TTT GGC CTC GAT GGG TCC TTC GTC TCA      1749
Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val Ser
    530                 535                 540

GAA AAG ACC TTT GAA ACA CTC TGC ACG GTC CGG ACC TGG ATT CTC ACC      1797
Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu Thr
545                 550                 555                 560

GTG GGC TAC ACA ACT GCC TTT GGG GCC ATG TTT GCA AAG ACC TGG AGG      1845
Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp Arg
                565                 570                 575

GTC CAT GCC ATC TTC AAA AAT GTG AAG ATG AAG AAG AAG ATC ATC AAA      1893
Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile Lys
            580                 585                 590

GAC CAG AAG CTG CTT GTG ATT GTG GGG GGC ATG CTG CTC ATC GAC CTG      1941
Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu
        595                 600                 605

TGC ATC CTG ATC TGT TGG CAG GCT GTG GAC CCC CTG CGG AGG ACA GTG      1989
Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr Val
    610                 615                 620

GAG AGG TAC AGC ATG GAG CCG GAC CCA GCA GGC CGG GAC ATC TCC ATC      2037
Glu Arg Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser Ile
625                 630                 635                 640
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CGC CCA TTG CTG GAA CAC TGC GAA AAC ACC CAC ATG ACC ATC TGG CTT      2085
Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp Leu
                645                 650                 655

GGC ATT GTC TAC GCC TAC AAG GGG CTC CTC ATG CTA TTC GGT TGT TTC      2133
Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys Phe
            660                 665                 670

TTG GCA TGG GAA ACC CGC AAT GTG AGC ATC CCT GCC CTC AAC GAC AGC      2181
Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp Ser
        675                 680                 685

AAG TAC ATC GGC ATG AGT GTG TAC AAT GTG GGG ATC ATG TGC ATC ATC      2229
Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile Ile
    690                 695                 700

GGG GCT GCT GTC TCC TTC CTG ACG CGT GAC CAG CCC AAC GTG CAG TTC      2277
Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln Phe
705                 710                 715                 720

TGC ATC GTG GCC CTG GTC ATC ATC TTC TGC AGC ACC ATC ACT CTC TGC      2325
Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu Cys
                725                 730                 735

CTG GTG TTT GTG CCA AAG CTC ATC ACT CTG AGG ACA AAC CCT GAC GCA      2373
Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp Ala
            740                 745                 750

GCC ACT CAG AAC AGG CGG TTC CAG TTC ACA CAG AAC CAG AAG AAA GAA      2421
Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys Glu
        755                 760                 765

GAT TCG AAG ACC TCC ACT TCA GTC ACC AGC GTG AAC CAG GCG AGC ACG      2469
Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser Thr
    770                 775                 780

TCA CGC CTG GAG GGA CTG CAG TCA GAA AAC CAC CGC CTT CGA ATG AAG      2517
Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met Lys
785                 790                 795                 800

ATC ACA GAG CTG GAC AAA GAC TTG GAA GAA GTC ACC ATG CAG CTA CAA      2565
Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu Gln
                805                 810                 815

GAC ACA CCA GAG AAG ACC ACA TAC ATC AAA CAG AAT CAC TAC CAA GAG      2613
Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln Glu
            820                 825                 830

CTC AAC GAC ATC CTC AGC TTG GGC AAC TTC ACA GAG AGC ACA GAT GGA      2661
Leu Asn Asp Ile Leu Ser Leu Gly Asn Phe Thr Glu Ser Thr Asp Gly
        835                 840                 845
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GGA AAG GCC ATT CTA AAA AAT CAC CTC GAT CAA AAC CCC CAG CTC CAG      2709
Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu Gln
    850                 855                 860

TGG AAC ACG ACA GAG CCC TCA AGA ACA TGC AAA GAC CCC ATA GAA GAC      2757
Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu Asp
865                 870                 875                 880

ATC AAC TCC CCG GAG CAC ATC CAG CGC CGG CTG TCG CTC CAG CTC CCC      2805
Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu Pro
                885                 890                 895

ATC CTT CAC CAC GCC TAC CTC CCA TCC ATC GGA GGC GTG GAT GCC AGC      2853
Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala Ser
            900                 905                 910

TGC GTC AGC CCC TGT GTC AGC CCT ACC GCC AGC CCT CGC CAC AGA CAC      2901
Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg His
        915                 920                 925

GTA CCA CCC TCC TTC CGA GTC ATG GTC TCG GGC CTG TAGGGGTGGG           2947
Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu
    930                 935                 940

AGGCCTGGGG CCGGGGCCTC CCCGGGACAG CACCATGCTG GGCCAAGGCG CCTGCCACAG    3007

GCACACTGAC GGCGGCGAGA AGCTGGGCAC CATGCTGCCT CTCCAGACTG CTGGAATGGC    3067

GCTCAGGCAG AGCGGGACTC GGCACCGACC TCGAGCCTTA TCTGTGAAGG TCTTACTCTC    3127

ACAGAGGAGA GGAATGACAA TGACTTCTCC TTCTTGGCGT CTGCAAACAA AGAGGAGTTG    3187

GGATGTCTGA AACTTGCAAA AACAAATCAA ACTCTAGACA AAGGAGAGAG GCCTCGGACT    3247

CCTGCTGTCC TCGCCAAGTG GCCAGAGCAA GGGCTCTGCA G                        3288
```

(2) INFORMATION ZU SEQ ID NO: 2:

(i) SEQUENZ CHARAKTERISTIKA:
(A) LÄNGE: 940 Aminosäuren
(B) ART: Aminosäure
(D) TOPOLOGIE: linear

(ii) ART DES MOLEKÜLS: Protein

(xi) SEQUENZBESCHREIBUNG: SEQ ID NO: 2:

```
Met Ala Ser Pro Pro Ser Ser Gly Gln Pro Arg Pro Pro Pro Pro Pro
 1               5                  10                  15

Pro Pro Pro Ala Arg Leu Leu Leu Pro Leu Leu Leu Ser Leu Leu Leu
            20                  25                  30
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Trp Leu Ala Pro Gly Ala Trp Gly Trp Thr Arg Gly Ala Pro Arg Pro
        35                  40                  45

Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu Thr
    50                  55                  60

Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala Val
65                  70                  75                  80

Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro Tyr
                85                  90                  95

Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys Gly
            100                 105                 110

Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu Met
        115                 120                 125

Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu Ser
    130                 135                 140

Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr Pro
145                 150                 155                 160

Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val Pro
                165                 170                 175

Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His Phe
            180                 185                 190

Arg Trp Arg Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe Ser
        195                 200                 205

Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile Glu
    210                 215                 220

Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val Lys
225                 230                 235                 240

Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp Gln
                245                 250                 255

Asn Met Ala Ala Lys Val Phe Cys Cys Ala Phe Glu Glu Ser Met Phe
            260                 265                 270

Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ala Trp
        275                 280                 285

Trp Glu Gln Val His Val Glu Ala Asn Ser Ser Arg Cys Leu Arg Arg
    290                 295                 300

Ser Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu Pro
305                 310                 315                 320
```

Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln Gln
325 330 335

Tyr Glu Arg Glu Tyr Asn Ser Lys Arg Ser Gly Val Gly Pro Ser Lys
340 345 350

Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr Leu
355 360 365

Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg Ile
370 375 380

Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Lys Ile Ile Leu Asn
385 390 395 400

Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val Phe
405 410 415

Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln Asp
420 425 430

Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr Leu
435 440 445

Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro Lys
450 455 460

Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro Leu
465 470 475 480

Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala Ser
485 490 495

Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile Lys
500 505 510

Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met Leu
515 520 525

Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val Ser
530 535 540

Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu Thr
545 550 555 560

Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp Arg
565 570 575

Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile Lys
580 585 590

Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu
595 600 605

Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr Val
610 615 620

Glu Arg Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser Ile
625 630 635 640

Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp Leu
645 650 655

Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys Phe
660 665 670

Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp Ser
675 680 685

Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile Ile
690 695 700

Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln Phe
705 710 715 720

Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu Cys
725 730 735

Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp Ala
740 745 750

Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys Glu
755 760 765

Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser Thr
770 775 780

Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met Lys
785 790 795 800

Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu Gln
805 810 815

Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln Glu
820 825 830

Leu Asn Asp Ile Leu Ser Leu Gly Asn Phe Thr Glu Ser Thr Asp Gly
835 840 845

Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu Gln
850 855 860

Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu Asp
865 870 875 880

Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu Pro
885 890 895

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Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala Ser
          900                 905                 910

Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg His
       915                 920                 925

Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu
   930                 935                 940
```

(2) INFORMATION ZU SEQ ID NO: 3:

(i) SEQUENZ CHARAKTERISTIKA:
(A) LÄNGE: 2826 Basenpaare
(B) ART: Nukleinsäure
(C) STRANGFORM: Einzel
(D) TOPOLOGIE: linear

(ii) ART DES MOLEKÜLS: cDNS zu mRNS

(iii) HYPOTHETISCH: NEIN

(iii) ANTISENSE: NEIN

(vi) URSPRÜNLICHE HERKUNFT:
(A) ORGANISMUS: Homo sapiens

(ix) MERKMALE:
(A) NAME/SCHLÜSSEL: CDS
(B) LAGE: 1..2826

(xi) SEQUENZBESCHREIBUNG: SEQ ID NO: 3:

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ATG GCT TCC CCG CGG AGC TCC GGG CAG CCC GGG CCG CCG CCG CCG CCG      48
Met Ala Ser Pro Arg Ser Ser Gly Gln Pro Gly Pro Pro Pro Pro Pro
 1               5                  10                  15

CCA CCG CCG CCC GCG CGC CTG CTA CTG CTA CTG CTG CTG CCG CTG CTG      96
Pro Pro Pro Pro Ala Arg Leu Leu Leu Leu Leu Leu Pro Leu Leu
            20                  25                  30

CTG CCT CTG GCG CCC GGG GCC TGG GGC TGG GCG CGG GGC GCC CCC CGG     144
Leu Pro Leu Ala Pro Gly Ala Trp Gly Trp Ala Arg Gly Ala Pro Arg
        35                  40                  45

CCG CCG CCC AGC AGC CCG CCG CTC TCC ATC ATG GGC CTC ATG CCG CTC     192
Pro Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu
    50                  55                  60

ACC AAG GAG GTG GCC AAG GGC AGC ATC GGG CGC GGT GTG CTC CCC GCC     240
Thr Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala
65                  70                  75                  80
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GTG GAA CTG GCC ATC GAG CAG ATC CGC AAC GAG TCA CTC CTG CGC CCC      288
Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro
                85                  90                  95

TAC TTC CTC GAC CTG CGG CTC TAT GAC ACG GAG TGC GAC AAC GCA AAA      336
Tyr Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys
            100                 105                 110

GGG TTG AAA GCC TTC TAC GAT GCA ATA AAA TAC GGG CCG AAC CAC TTG      384
Gly Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu
        115                 120                 125

ATG GTG TTT GGA GGC GTC TGT CCA TCC GTC ACA TCC ATC ATT GCA GAG      432
Met Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu
    130                 135                 140

TCC CTC CAA GGC TGG AAT CTG GTG CAG CTT TCT TTT GCT GCA ACC ACG      480
Ser Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr
145                 150                 155                 160

CCT GTT CTA GCC GAT AAG AAA AAA TAC CCT TAT TTC TTT CGG ACC GTC      528
Pro Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val
                165                 170                 175

CCA TCA GAC AAT GCG GTG AAT CCA GCC ATT CTG AAG TTG CTC AAG CAC      576
Pro Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His
            180                 185                 190

TAC CAG TGG AAG CGC GTG GGC ACG CTG ACG CAA GAC GTT CAG AGG TTC      624
Tyr Gln Trp Lys Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe
        195                 200                 205

TCT GAG GTG CGG AAT GAC CTG ACT GGA GTT CTG TAT GGC GAG GAC ATT      672
Ser Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile
    210                 215                 220

GAG ATT TCA GAC ACC GAG AGC TTC TCC AAC GAT CCC TGT ACC AGT GTC      720
Glu Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val
225                 230                 235                 240

AAA AAG CTG AAG GGG AAT GAT GTG CGG ATC ATC CTT GGC CAG TTT GAC      768
Lys Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp
                245                 250                 255

CAG AAT ATG GCA GCA AAA GTG TTC TGT TGT GCA TAC GAG GAG AAC ATG      816
Gln Asn Met Ala Ala Lys Val Phe Cys Cys Ala Tyr Glu Glu Asn Met
            260                 265                 270

TAT GGT AGT AAA TAT CAG TGG ATC ATT CCG GGC TGG TAC GAG CCT TCT      864
Tyr Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ser
        275                 280                 285
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TGG TGG GAG CAG GTG CAC ACG GAA GCC AAC TCA TCC CGC TGC CTC CGG      912
Trp Trp Glu Gln Val His Thr Glu Ala Asn Ser Ser Arg Cys Leu Arg
    290                 295                 300

AAG AAT CTG CTT GCT GCC ATG GAG GGC TAC ATT GGC GTG GAT TTC GAG      960
Lys Asn Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu
305                 310                 315                 320

CCC CTG AGC TCC AAG CAG ATC AAG ACC ATC TCA GGA AAG ACT CCA CAG     1008
Pro Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln
                325                 330                 335

CAG TAT GAG AGA GAG TAC AAC AAC AAG CGG TCA GGC GTG GGG CCC AGC     1056
Gln Tyr Glu Arg Glu Tyr Asn Asn Lys Arg Ser Gly Val Gly Pro Ser
            340                 345                 350

AAG TTC CAC GGG TAC GCC TAC GAT GGC ATC TGG GTC ATC GCC AAG ACA     1104
Lys Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr
        355                 360                 365

CTG CAG AGG GCC ATG GAG ACA CTG CAT GCC AGC AGC CGG CAC CAG CGG     1152
Leu Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg
    370                 375                 380

ATC CAG GAC TTC AAC TAC ACG GAC CAC ACG CTG GGC AGG ATC ATC CTC     1200
Ile Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Arg Ile Ile Leu
385                 390                 395                 400

AAT GCC ATG AAC GAG ACC AAC TTC TTC GGG GTC ACG GGT CAA GTT GTA     1248
Asn Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val
                405                 410                 415

TTC CGG AAT GGG GAG AGA ATG GGG ACC ATT AAA TTT ACT CAA TTT CAA     1296
Phe Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln
            420                 425                 430

GAC AGC AGG GAG GTG AAG GTG GGA GAG TAC AAC GCT GTG GCC GAC ACA     1344
Asp Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr
        435                 440                 445

CTG GAG ATC ATC AAT GAC ACC ATC AGG TTC CAA GGA TCC GAA CCA CCA     1392
Leu Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro
    450                 455                 460

AAA GAC AAG ACC ATC ATC CTG GAG CAG CTG CGG AAG ATC TCC CTA CCT     1440
Lys Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro
465                 470                 475                 480

CTC TAC AGC ATC CTC TCT GCC CTC ACC ATC CTC GGG ATG ATC ATG GCC     1488
Leu Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala
                485                 490                 495
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AGT GCT TTT CTC TTC TTC AAC ATC AAG AAC CGG AAT CAG AAG CTC ATA      1536
Ser Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile
            500                 505                 510

AAG ATG TCG AGT CCA TAC ATG AAC AAC CTT ATC ATC CTT GGA GGG ATG      1584
Lys Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met
        515                 520                 525

CTC TCC TAT GCT TCC ATA TTT CTC TTT GGC CTT GAT GGA TCC TTT GTC      1632
Leu Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val
    530                 535                 540

TCT GAA AAG ACC TTT GAA ACA CTT TGC ACC GTC AGG ACC TGG ATT CTC      1680
Ser Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu
545                 550                 555                 560

ACC GTG GGC TAC ACG ACC GCT TTT GGG GCC ATG TTT GCA AAG ACC TGG      1728
Thr Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp
                565                 570                 575

AGA GTC CAC GCC ATC TTC AAA AAT GTG AAA ATG AAG AAG AAG ATC ATC      1776
Arg Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile
            580                 585                 590

AAG GAC CAG AAA CTG CTT GTG ATC GTG GGG GGC ATG CTG CTG ATC GAC      1824
Lys Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp
        595                 600                 605

CTG TGT ATC CTG ATC TGC TGG CAG GCT GTG GAC CCC CTG CGA AGG ACA      1872
Leu Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr
    610                 615                 620

GTG GAG AAG TAC AGC ATG GAG CCG GAC CCA GCA GGA CGG GAT ATC TCC      1920
Val Glu Lys Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser
625                 630                 635                 640

ATC CGC CCT CTC CTG GAG CAC TGT GAG AAC ACC CAT ATG ACC ATC TGG      1968
Ile Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp
                645                 650                 655

CTT GGC ATC GTC TAT GCC TAC AAG GGA CTT CTC ATG TTG TTC GGT TGT      2016
Leu Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys
            660                 665                 670

TTC TTA GCT TGG GAG ACC CGC AAC GTC AGC ATC CCC GCA CTC AAC GAC      2064
Phe Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp
        675                 680                 685

AGC AAG TAC ATC GGG ATG AGT GTC TAC AAC GTG GGG ATC ATG TGC ATC      2112
Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile
    690                 695                 700
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ATC GGG GCC GCT GTC TCC TTC CTG ACC CGG GAC CAG CCC AAT GTG CAG      2160
Ile Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln
705                 710                 715                 720

TTC TGC ATC GTG GCT CTG GTC ATC ATC TTC TGC AGC ACC ATC ACC CTC      2208
Phe Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu
                725                 730                 735

TGC CTG GTA TTC GTG CCG AAG CTC ATC ACC CTG AGA ACA AAC CCA GAT      2256
Cys Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp
            740                 745                 750

GCA GCA ACG CAG AAC AGG CGA TTC CAG TTC ACT CAG AAT CAG AAG AAA      2304
Ala Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys
        755                 760                 765

GAA GAT TCT AAA ACG TCC ACC TCG GTC ACC AGT GTG AAC CAA GCC AGC      2352
Glu Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser
    770                 775                 780

ACA TCC CGC CTG GAG GGC CTA CAG TCA GAA AAC CAT CGC CTG CGA ATG      2400
Thr Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met
785                 790                 795                 800

AAG ATC ACA GAG CTG GAT AAA GAC TTG GAA GAG GTC ACC ATG CAG CTG      2448
Lys Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu
                805                 810                 815

CAG GAC ACA CCA GAA AAG ACC ACC TAC ATT AAA CAG AAC CAC TAC CAA      2496
Gln Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln
            820                 825                 830

GAG CTC AAT GAC ATC CTC AAC CTG GGA AAC TTC ACT GAG AGC ACA GAT      2544
Glu Leu Asn Asp Ile Leu Asn Leu Gly Asn Phe Thr Glu Ser Thr Asp
        835                 840                 845

GGA GGA AAG GCC ATT TTA AAA AAT CAC CTC GAT CAA AAT CCC CAG CTA      2592
Gly Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu
    850                 855                 860

CAG TGG AAC ACA ACA GAG CCC TCT CGA ACA TGC AAA GAT CCT ATA GAA      2640
Gln Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu
865                 870                 875                 880

GAT ATA AAC TCT CCA GAA CAC ATC CAG CGT CGG CTG TCC CTC CAG CTC      2688
Asp Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu
                885                 890                 895

CCC ATC CTC CAC CAC GCC TAC CTC CCA TCC ATC GGA GGC GTG GAC GCC      2736
Pro Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala
            900                 905                 910
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AGC TGT GTC AGC CCC TGC GTC AGC CCC ACC GCC AGC CCC CGC CAC AGA      2784
Ser Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg
        915                 920                 925

CAT GTG CCA CCC TCC TTC CGA GTC ATG GTC TCG GGC CTG TAA              2826
His Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu
    930                 935                 940
```

(2) INFORMATION ZU SEQ ID NO: 4:

(i) SEQUENZ CHARAKTERISTIKA:
(A) LÄNGE: 941 Aminosäuren
(B) ART: Aminosäure
(D) TOPOLOGIE: linear

(ii) ART DES MOLEKÜLS: Protein

(xi) SEQUENZBESCHREIBUNG: SEQ ID NO: 4:

```
Met Ala Ser Pro Arg Ser Ser Gly Gln Pro Gly Pro Pro Pro Pro Pro
 1               5                  10                  15

Pro Pro Pro Pro Ala Arg Leu Leu Leu Leu Leu Leu Leu Pro Leu Leu
            20                  25                  30

Leu Pro Leu Ala Pro Gly Ala Trp Gly Trp Ala Arg Gly Ala Pro Arg
        35                  40                  45

Pro Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu
    50                  55                  60

Thr Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala
65                  70                  75                  80

Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro
                85                  90                  95

Tyr Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys
            100                 105                 110

Gly Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu
        115                 120                 125

Met Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu
    130                 135                 140

Ser Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr
145                 150                 155                 160

Pro Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val
                165                 170                 175

Pro Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His
            180                 185                 190
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Tyr Gln Trp Lys Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe
195 200 205

Ser Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile
210 215 220

Glu Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val
225 230 235 240

Lys Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp
245 250 255

Gln Asn Met Ala Ala Lys Val Phe Cys Cys Ala Tyr Glu Glu Asn Met
260 265 270

Tyr Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ser
275 280 285

Trp Trp Glu Gln Val His Thr Glu Ala Asn Ser Ser Arg Cys Leu Arg
290 295 300

Lys Asn Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu
305 310 315 320

Pro Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln
325 330 335

Gln Tyr Glu Arg Glu Tyr Asn Asn Lys Arg Ser Gly Val Gly Pro Ser
340 345 350

Lys Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr
355 360 365

Leu Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg
370 375 380

Ile Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Arg Ile Ile Leu
385 390 395 400

Asn Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val
405 410 415

Phe Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln
420 425 430

Asp Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr
435 440 445

Leu Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro
450 455 460

Lys Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro
465 470 475 480

Leu Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala
485 490 495

Ser Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile
500 505 510

Lys Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met
515 520 525

Leu Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val
530 535 540

Ser Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu
545 550 555 560

Thr Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp
565 570 575

Arg Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile
580 585 590

Lys Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp
595 600 605

Leu Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr
610 615 620

Val Glu Lys Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser
625 630 635 640

Ile Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp
645 650 655

Leu Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys
660 665 670

Phe Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp
675 680 685

Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile
690 695 700

Ile Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln
705 710 715 720

Phe Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu
725 730 735

Cys Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp
740 745 750

Ala Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys
755 760 765

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Glu Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser
    770                 775                 780

Thr Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met
785                 790                 795                 800

Lys Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu
                805                 810                 815

Gln Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln
            820                 825                 830

Glu Leu Asn Asp Ile Leu Asn Leu Gly Asn Phe Thr Glu Ser Thr Asp
        835                 840                 845

Gly Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu
    850                 855                 860

Gln Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu
865                 870                 875                 880

Asp Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu
                885                 890                 895

Pro Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala
            900                 905                 910

Ser Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg
        915                 920                 925

His Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu
    930                 935                 940
```

Patentansprüche

1. Proteinheteromer, enthaltend mindestens ein $GABA_B$-Rezeptorprotein und mindestens ein Protein mit der in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellte Aminosäuresequenz oder eine daraus durch Substitution, Inversion, Insertion oder Deletion von einem oder mehreren Aminosäureresten erhältliche Sequenz, wobei wenigstens noch eine der wesentlichen biologischen Eigenschaften des in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Proteins oder des Proteinheteromer erhalten bleibt.
2. Isoliertes Protein, enthaltend die in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellte Aminosäuresequenz oder eine daraus durch Substitution, Inversion, Insertion oder Deletion von einem oder mehreren Aminosäureresten erhältliche Sequenz, wobei wenigstens noch eine der wesentlichen biologischen Eigenschaften des in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Proteins erhalten bleibt.
3. Nukleinsäuresequenz codierend für ein Protein gemäß Anspruch 2.
4. Nukleinsäuresequenz nach Anspruch 3, dadurch gekennzeichnet, daß sie für ein Protein codiert, das wenigstens 60% Identität mit der in SEQ ID NO: 2 oder SEQ ID NO: 4 dargestellten Sequenz hat.
5. Nukleinsäuresequenz nach Anspruch 3, dadurch gekennzeichnet, daß sie die in SEQ ID NO: 1 oder SEQ ID NO: 3 dargestellte Sequenz enthält.
6. Rekombinantes Nukleinsäurekonstrukt, enthaltend eine Nukleinsäuresequenz gemäß Anspruch 3 oder eine Nukleinsäuresequenz gemäß Anspruch 3 und eine Sequenz, die für ein $GABA_B$-Rezeptorprotein kodiert, funktionell verknüpft mit mindestens einem genetischen Regulationselement.
7. Wirtsorganismus, transformiert mit einer Nukleinsäuresequenz gemäß Anspruch 3 oder einem rekombinanten Nukleinsäurekonstrukt gemäß Anspruch 6 oder mit einer Nukleinsäuresequenz gemäß Anspruch 3 oder einem rekombinanten Nukleinsäurekonstrukt gemäß Anspruch 6 zusammen mit einer Sequenz, die für ein $GABA_B$-Rezeptorprotein kodiert.
8. Wirtsorganismus, transformiert mit einem rekombinanten Nukleinsäurekonstrukt gemäß Anspruch 6.
9. Transgene Tiere enthaltend eine funktionelle oder nicht funktionelle Nukleinsäuresequenz gemäß den Ansprüchen 3 bis 5 oder ein funktionelles oder nicht funktionelles Nukleinsäurekonstrukt nach Anspruch 6.
10. Transgene Tiere, in dessen Keimzellen oder der Gesamtheit oder einem Teil der somatischen Zellen, oder in

dessen Keimzellen und der Gesamtheit oder einem Teil der somatischen Zellen die Nukleotidsequenz gemäß Anspruch 3 durch gentechnische Verfahren verändert oder durch Einfügen von DNA-Elementen unterbrochen wurde.

11. Verwendung einer Nukleinsäuresequenz gemäß Anspruch 3, eines Nukleinsäurekonstrukts gemäß Anspruch 6, eines Proteinheteromeren gemäß Anspruch 1 oder Proteins gemäß Anspruch 2 zur Identifizierung von Proteinen, die zu einem Proteinheteromeren gemäß Anspruch 1 oder einem Protein gemäß Anspruch 2 spezifische Bindungsaffinitäten aufweisen, oder zur Identifizierung von Nukleinsäuren, die für Proteine kodieren, die zu einem Proteinheteromeren gemäß Anspruch 1 oder einem Protein gemäß Anspruch 2 spezifische Bindungsaffinitäten aufweisen.

12. Verwendung des two-hybrid Systems oder biochemischer Verfahren zur Identifizierung der Interaktionsdomänen von metabotropen Rezeptoren und Verwendung zur pharmakotherapeutischen Intervention.

13. Verwendung der aus einer Strukturaufklärung eines Proteinheteromeren gemäß Anspruch 1 oder eines Proteins gemäß Anspruch 2 resultierenden Information zum gezielten Auffinden oder zur gezielten Herstellung von Substanzen mit spezifischer Bindungsaktivität zu einem Proteinheteromeren gemäß Anspruch 1 oder einem Protein gemäß Anspruch 2.

14. Verwendung eines Proteinheteromeren gemäß Anspruch 1 oder eines Proteins gemäß Anspruch 2 oder Peptidfragmenten davon als Antigen zur Erzeugung von spezifischen mono- oder polyklonalen Antikörpern oder Antikörpergemischen gerichtet gegen Proteine gemäß Anspruch 1 oder 2.

15. Mono- oder polyklonale Antikörper oder Antikörpergemische, die spezifisch Proteine nach Anspruch 1 oder 2 erkennen.

16. Verwendung einer Nukleinsäuresequenz gemäß Anspruch 3 oder eines Fragmentes davon zur Isolierung einer genomischen Sequenz über Homologiescreening.

17. Verwendung einer Nukleinsäuresequenz gemäß Anspruch 3 als Marker für humane Erbkrankheiten.

18. Verwendung einer Nukleinsäuresequenz nach Anspruch 3 oder Teilen davon zur Gentherapie.

19. Verwendung einer zu der Nukleinsäuresequenz gemäß Anspruch 3 oder zu Teilen von ihr komplementären Nukleinsäuresequenz zur Gentherapie.

20. Verfahren zum Auffinden von Substanzen mit spezifischer Bindungsaffinität zu einem Protein nach Anspruch 1 oder 2, das folgende Schritte umfaßt:

a) Inkubation des Proteins gemäß Anspruch 1 oder 2 mit der zu testenden Substanz.

b) Detektion der Bindung der zu testenden Substanz an das Protein.

21. Verfahren nach Anspruch 20, dadurch gekennzeichnet, daß die Detektion der Bindung durch Messen der Antagonisierung oder Agonisierung der $GABA_B$-Rezeptor-Aktivität erfolgt.

22. Verfahren nach Anspruch 20, dadurch gekennzeichnet, daß die Detektion der Bindung von Substanzen an ein Protein gemäß Anspruch 1 durch Messen einer physiologischen Wirkung, wie z. B. einer Änderung der Calcium-, cAMP-, IP3-Konzentration oder des Membranpotentials, erfolgt.

23. Verfahren zum qualitativen oder quantitativen Nachweis einer Nukleinsäure gemäß Anspruch 3 in einer biologischen Probe, das einen oder mehrere der folgenden Schritte umfaßt:

a) Inkubation einer biologischen Probe mit einer bekannten Menge an Nukleinsäure gemäß Anspruch 3 oder einer bekannten Menge an Oligonukleotiden, die als Primer für eine Amplifikation der Nukleinsäure gemäß Anspruch 3 geeignet sind oder Mischungen davon,

b) Nachweis der Nukleinsäure gemäß Anspruch 3 durch spezifische Hybridisierung oder PCR-Amplifikation,

c) Vergleich der Menge an hybridisierender Nukleinsäure gemäß Anspruch 3 oder an durch PCR Amplifikation gewonnener Nukleinsäure gemäß Anspruch 3 mit einem Standard.

24. Verfahren zum qualitativen und quantitativen Nachweis eines Proteins gemäß Anspruch 1 oder 2 in einer biologischen Probe, das einen oder mehrere der folgenden Schritte umfaßt:

a) Inkubation einer biologischen Probe mit einem Antikörper gemäß Anspruch 15, der spezifisch gegen Proteine gemäß Anspruch 1 oder 2 gerichtet ist,

b) Nachweis des Antikörper/Antigenkomplexes,

c) Vergleich der Mengen des Antikörper/Antigenkomplexes mit einem Mengenstandard.

25. Verfahren zum Auffinden von Substanzen, die spezifisch an ein Protein mit einer Aminosäuresequenz gemäß Anspruch 2 binden, das einen oder mehrere der folgenden Schritte umfaßt:

a) Expression des Proteins in eukaryotischen oder prokaryotischen Zellen.

b) Inkubation des Proteins mit den zu testenden Substanzen.

c) Nachweis der Bindung einer Substanz an den Rezeptor bzw. eines Effektes auf die Rezeptorfunktion.

26. Verfahren zum Auffinden von Substanzen, die spezifisch an ein Protein mit einer Aminosäuresequenz gemäß Anspruch 2 bzw. an eine Nukleinsäuresequenz gemäß Anspruch 3 binden und dadurch hemmende oder aktivierende funktionelle Effekte auf die GABAerge Signalweiterleitung in zentralnervösen Neuronen hervorrufen.

27. Verfahren zum Auffinden von Substanzen, die die Interaktion von Proteinen mit Aminosäuresequenzen gemäß Anspruch 2 mit anderen metabotropen Rezeptoren hemmen oder verstärken.

28. Verfahren zum Auffinden von Substanzen, die die Interaktion von Liganden mit dem Proteinheteromer gemäß Anspruch 1 oder Proteinen mit Aminosäuresequenzen gemäß Anspruch 2 hemmen oder verstärken.

29. Verfahren zum Auffinden von Substanzen, die die Interaktion von Proteinen mit Aminosäuresequenzen gemäß Anspruch 2 mit G-Proteinen oder anderen Signaltransduktionsmolekülen hemmen oder verstärken.

30. Verfahren zur qualitativen und quantitativen Bestimmung von Proteinen gemäß Anspruch 2 unter Benutzung von spezifischen Agonisten oder Antagonisten.

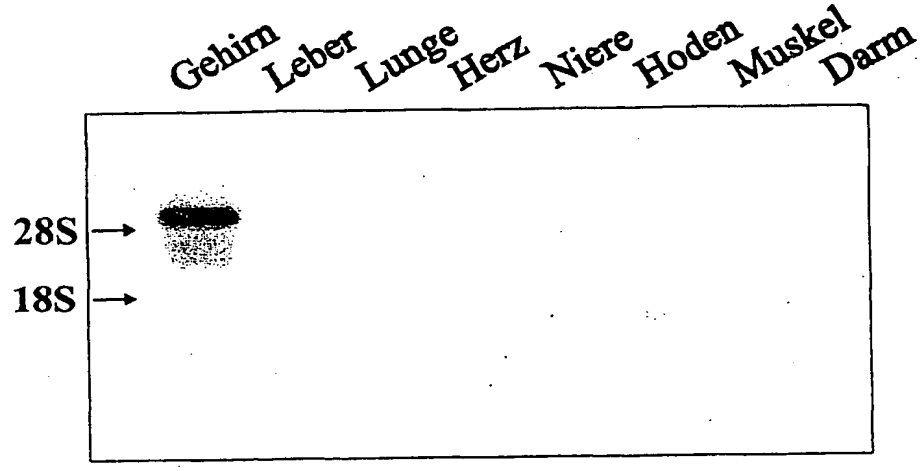
31. Verfahren zur Quantifizierung der Proteinaktivität eines Proteins gemäß Anspruch 2.

Hierzu 2 Seite(n) Zeichnungen

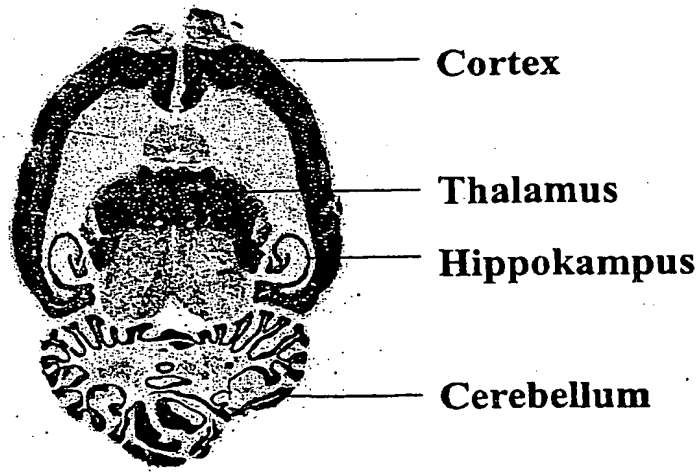
Numme DE 198 41 941 A1
Int. Cl.[7]: C 07 K 16/00
Offenlegungstag: 16. März 2000

Figur 1

A)



B)



Nr DE 198 41 941 A1
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Figur 2

cells, and new and useful methods for cultured growth of such host cells capable of expression of the polypeptide and related products.

[0132] Vectors which comprise the isolated nucleic acid molecule described hereinabove also are provided. Suitable vectors comprise, but are not limited to, a plasmid or a virus. These vectors may be transformed into a suitable host cell to form a host cell expression system for the production of a $GABA_BR2$ polypeptide. Suitable host cells include, for example, neuronal cells such as the glial cell line C6, a Xenopus cell such as an oocyte or melanophore cell, as well as numerous mammalian cells and non-neuronal cells.

[0133] This invention further provides for any vector or plasmid which comprises modified untranslated sequences, which are beneficial for expression in desired host cells or for use in binding or functional assays. For example, a vector or plasmid with untranslated sequences of varying lengths may express differing amounts of the polypeptide depending upon the host cell used. In an embodiment, the vector or plasmid comprises the coding sequence of the polypeptide and the regulatory elements necessary for expression in the host cell.

[0134] As used herein, the phrase "specifically hybridizing" means the ability of a nucleic acid molecule to recognize a nucleic acid sequence complementary to its own and to form double-helical segments through hydrogen bonding between complementary base pairs. The term "complementary" is used in its usual sense in the art, i.e., G and C are complementary and A is complementary to T (or U in RNA), such that two strands of nucleic acid are "complementary" only if every base matches the opposing base exactly.

[0135] This invention is directed to a purified $GABA_BR2$ protein.

[0136] This invention is directed to a vector comprising a above-identified nucleic acid.

[0137] In one embodiment, the vector is adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the nucleic acid in the bacterial cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

[0138] In another embodiment, the vector is adapted for expression in an amphibian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the amphibian cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

[0139] In another embodiment, the vector is adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the nucleic acid in the yeast cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

[0140] In another embodiment, the vector is adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the nucleic acid in the insect cell operatively linked to the nucleic acid encoding the $GABA_BR2$ polypeptide so as to permit expression thereof.

[0141] In one embodiment, the vector is a baculovirus.

[0142] In another embodiment, the vector is adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

[0143] In one embodiment, the vector is a plasmid.

[0144] In a further embodiment, the plasmid is designated BO-55 (ATCC Accession No. 209104).

[0145] In a further embodiment, the plasmid is designated pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

[0146] This invention provides a plasmid designated pEXJT3T7-hGABAB2 (ATCC Accession No. 203515) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the human polypeptide so as to permit expression thereof.

[0147] This plasmid (pEXJT3T7-hGABAB2) was deposited on Dec. 9, 1998, with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Va. 20110-2209, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 203515.

[0148] This invention provides a plasmid designated BO-55 (ATCC Accession No. 209104) which comprises the regulatory elements necessary for expression of DNA in a mammalian cell operatively linked to DNA encoding the rat polypeptide so as to permit expression thereof.

[0149] This plasmid (BO-55) was deposited on Jun. 10, 1997, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md. 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 209104.

[0150] Nucleic acid probe technology is well known to those skilled in the art who will readily appreciate that such probes may vary greatly in length and may be labeled with a detectable label, such as a radioisotope or fluorescent dye, to facilitate detection of the probe. DNA probe molecules may be produced by insertion of a DNA molecule which encodes the polypeptides of this invention into suitable vectors, such as plasmids or bacteriophages, followed by transforming into suitable bacterial host cells, replication in the transformed bacterial host cells and harvesting of the DNA probes, using methods well known in the art. Alternatively, probes may be generated chemically from DNA synthesizers.

[0151] RNA probes may be generated by inserting the DNA molecule which encodes the polypeptides of this invention downstream of a bacteriophage promoter such as T3, T7 or SP6. Large amounts of RNA probe may be produced by incubating the labeled nucleotides with the linearized fragment where it contains an upstream promoter in the presence of the appropriate RNA polymerase.

[0152] This invention is directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe compris-

ing at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within one of the two strands of the nucleic acid encoding the $GABA_BR2$ polypeptide contained in plasmid BO-55, and detecting hybridization of the probe to the nucleic acid.

[0153] This invention is directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the nucleic acid sequence shown in FIGS. 22A-22D (SEQ ID NO:46) or (b) the reverse complement to the nucleic acid sequence shown in FIGS. 22A-22D (SEQ ID NO:46), and detecting hybridization of the probe to the nucleic acid.

[0154] This invention is directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within one of the two strands of the nucleic acid encoding the $GABA_BR2$ polypeptide contained in plasmid pEXJT3T7-hGABAB2 and detecting hybridization of the probe to the nucleic acid.

[0155] This invention is directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the nucleic acid sequence shown in FIGS. 3A-3D (SEQ ID NO:3) or (b) the reverse complement to the nucleic acid sequence shown in FIGS. 3A-3D (SEQ ID NO:3), and detecting hybridization of the probe to the nucleic acid.

[0156] In one embodiment, the nucleic acid is DNA.

[0157] In another embodiment, the nucleic acid is RNA.

[0158] In one embodiment, the probe comprises at least 15 nucleotides complementary to a unique segment of the sequence of the nucleic acid molecule encoding the $GABA_BR2$ polypeptide.

[0159] This invention is directed to a method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising a nucleic acid of at least 15 nucleotides which is complementary to the antisense sequence of a unique segment of the sequence of the nucleic acid encoding the $GABA_BR2$ polypeptide, and detecting hybridization of the probe to the nucleic acid.

[0160] This invention is directed to a method of inhibiting translation of mRNA encoding a $GABA_BR2$ polypeptide which comprises contacting such mRNA with an antisense oligonucleotide having a sequence capable of specifically hybridizing to the above-identified mRNA, so as to prevent translation of the mRNA.

[0161] This invention is directed to a method of inhibiting translation of mRNA encoding a $GABA_BR2$ polypeptide which comprises contacting such mRNA with an antisense oligonucleotide having a sequence capable of specifically hybridizing to the above-identified genomic DNA.

[0162] In one embodiment, the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.

[0163] In another embodiment, the isolated antibody is capable of binding to a $GABA_BR2$ polypeptide encoded by an above-identified nucleic acid.

[0164] In another embodiment, the $GABA_BR2$ polypeptide is a human $GABA_BR2$ polypeptide.

[0165] This invention is directed to an antibody capable of competitively inhibiting the binding of an above-identified antibody to a $GABA_BR2$ polypeptide.

[0166] In one embodiment, the antibody is a monoclonal antibody.

[0167] In one embodiment, the monoclonal antibody is directed to an epitope of a $GABA_BR2$ polypeptide present on the surface of a $GABA_BR2$ polypeptide expressing cell.

[0168] In another embodiment, the oligonucleotide is coupled to a substance which inactivates mRNA.

[0169] In another embodiment, the substance which inactivates mRNA is a ribozyme.

[0170] This invention is directed to a pharmaceutical composition which comprises an amount of an above-identified antibody effective to block binding of a ligand to the $GABA_BR2$ polypeptide and a pharmaceutically acceptable carrier.

[0171] As used herein, "pharmaceutically acceptable carriers" means any of the standard pharmaceutically acceptable carriers. Examples include, but are not limited to, phosphate buffered saline, physiological saline, water and emulsions, such as oil/water emulsions.

[0172] Animal model systems which elucidate the physiological and behavioral roles of the polypeptides of this invention are produced by creating transgenic animals in which the activity of the polypeptide is either increased or decreased, or the amino acid sequence of the expressed polypeptide is altered, by a variety of techniques. Examples of these techniques include, but are not limited to: 1) Insertion of normal or mutant versions of DNA encoding the polypeptide, by microinjection, electroporation, retroviral transfection or other means well known to those skilled in the art, into appropriate fertilized embryos in order to produce a transgenic animal or 2) Homologous recombination of mutant or normal, human or animal versions of these genes with the native gene locus in transgenic animals to alter the regulation of expression or the structure of these polypeptide sequences. The technique of homologous recombination is well known in the art. It replaces the native gene with the inserted gene and so is useful for producing an animal that cannot express native polypeptides but does express, for example, an inserted mutant polypeptide, which has replaced the native polypeptide in the animal's genome by recombination, resulting in underexpression of the transporter. Microinjection adds genes to the genome, but does not remove them, and so is useful for producing an animal

which expresses its own and added polypeptides, resulting in overexpression of the polypeptides.

[0173] One means available for producing a transgenic animal, with a mouse as an example, is as follows: Female mice are mated, and the resulting fertilized eggs are dissected out of their oviducts. The eggs are stored in an appropriate medium such as M2 medium. DNA or cDNA encoding a polypeptide of this invention is purified from a vector by methods well known in the art. Inducible promoters may be fused with the coding region of the DNA to provide an experimental means to regulate expression of the trans-gene. Alternatively, or in addition, tissue specific regulatory elements may be fused with the coding region to permit tissue-specific expression of the trans-gene. The DNA, in an appropriately buffered solution, is put into a microinjection needle (which may be made from capillary tubing using a pipet puller) and the egg to be injected is put in a depression slide. The needle is inserted into the pronucleus of the egg, and the DNA solution is injected. The injected egg is then transferred into the oviduct of a pseudopregnant mouse (a mouse stimulated by the appropriate hormones to maintain pregnancy but which is not actually pregnant), where it proceeds to the uterus, implants, and develops to term. As noted above, microinjection is not the only method for inserting DNA into the egg cell, and is used here only for exemplary purposes.

[0174] This invention is directed to a transgenic, nonhuman mammal expressing DNA encoding a $GABA_nR2$ polypeptide.

[0175] This invention is directed to a transgenic, nonhuman mammal comprising a homologous recombination knockout of the native $GABA_BR2$ polypeptide.

[0176] This invention is further directed to a transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a $GABA_BR2$ polypeptide so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding such $GABA_BR2$ polypeptide and which hybridizes to such mRNA encoding such $GABA_BR2$ polypeptide, thereby reducing its translation.

[0177] This invention is directed to an above-identified transgenic, nonhuman mammal, wherein the DNA encoding the $GABA_BR2$ polypeptide additionally comprises an inducible promoter.

[0178] This invention is directed to an above-identified transgenic, nonhuman mammal, wherein the DNA encoding the $GABA_BR2$ polypeptide additionally comprises tissue specific regulatory elements.

[0179] This invention is directed to an above-identified transgenic, nonhuman mammal, wherein the transgenic, nonhuman mammal is a mouse.

[0180] This invention is directed to method of detecting the presence of a $GABA_BR2$ polypeptide on the surface of a cell which comprises contacting the cell with an above-identified antibody under conditions permitting binding of the antibody to the polypeptide, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a $GABA_BR2$ polypeptide on the surface of the cell.

[0181] This invention is directed to a method of preparing a purified $GABA_nR2$ polypeptide which comprises:

[0182] a. inducing cells to express a $GABA_BR2$ polypeptide;

[0183] b. recovering the polypeptide so expressed from the induced cells; and

[0184] c. purifying the polypeptide so recovered.

[0185] This invention is directed to a method of preparing the purified $GABA_BR2$ polypeptide which comprises:

[0186] a. inserting a nucleic acid encoding the $GABA_BR2$ polypeptide into a suitable vector;

[0187] b. introducing the resulting vector in a suitable host cell;

[0188] c. placing the resulting cell in suitable condition permitting the production of the $GABA_BR2$ polypeptide;

[0189] d. recovering the polypeptide produced by the resulting cell; and

[0190] e. isolating or purifying the polypeptide so recovered.

[0191] This invention is directed to a $GABA_BR1/R2$ receptor comprising two polypeptides, one of which is a $GABA_BR2$ polypeptide and another of which is a $GABA_BR1$ polypeptide.

[0192] This invention is directed to a method of forming a $GABA_BR1/R2$ receptor which comprises inducing cells to express both a $GABA_BR1$ polypeptide and a $GABA_BR2$ polypeptide.

[0193] $GABA_BR1$ as used in this application could be $GABA_BR1a$ or $GABA_BR1b$. The observation that at least two variants of the $GABA_BR1$ polypeptide exist raises the possibility that $GABA_BR2$ splice variants may exist or that there may exist introns in coding or non-coding regions of the genes encoding the $GABA_BR2$ polypeptides. In addition, spliced form(s) of mRNA may encode additional amino acids either upstream of the currently defined starting methionine or within the coding region. Further, the existence and use of alternative exons is possible, whereby the mRNA may encode different amino acids within the region comprising the exon. In addition, single amino acid substitutions may arise via the mechanism of RNA editing such that the amino acid sequence of the expressed protein is different than that encoded by the original gene (Burns et al., 1996; Chu et al., 1996). Such variants may exhibit pharmacologic properties differing from the polypeptide encoded by the original gene.

[0194] The activity of a G-protein coupled receptor (GPCR) typically is measured using any of a variety of functional assays in which activation of the receptor in question results in an observable change in the level of some second messenger system, including but not limited to adenylate cyclase, calcium mobilization, arachidonic acid release, ion channel activity, inositol phospholipid hydrolysis or guanylyl cyclase. Heterologous expression systems utilizing appropriate host cells to express the nucleic acids of the subject invention are used to obtain the desired second messenger coupling. Receptor activity may also be assayed in an oocyte expression system.

[0195] The pharmacologic properties of the receptor described herein when $GABA_BR2$ is co-expressed with

$GABA_BR1$, are similar to the pharmacologic properties of the $GABA_B$ receptor observed using tissues. For convenience, in the context of the present invention applicants will refer to the product of the heterologous coexpression of $GABA_BR2$ and $GABA_BR1$ as the "$GABA_BR1/R2$ receptor." Thus, a cell expressing nucleic acid encoding a $GABA_BR1/R2$ receptor is to be understood to refer to a cell expressing both nucleic acid encoding a $GABA_BR1$ polypeptide and nucleic acid encoding a $GABA_BR2$ polypeptide. In this application, $GABA_BR1$ can be either $GABA_BR1a$ or $GABA_BR1b$.

[0196] This invention is directed to an antibody capable of binding to a $GABA_BR1/R2$ receptor, wherein the $GABA_BR2$ polypeptide is encoded by an above-identified nucleic acid.

[0197] This invention is directed to an above-identified antibody, wherein the $GABA_BR2$ polypeptide is a human $GABA_BR2$ polypeptide.

[0198] This invention is directed to an antibody capable of competitively inhibiting the binding of an above-identified antibody to a $GABA_BR1/R2$ receptor.

[0199] In one embodiment, the antibody is a monoclonal antibody.

[0200] This invention is directed to an above-identified monoclonal antibody directed to an epitope of a $GABA_BR1/R2$ receptor present on the surface of a $GABA_BR1/R2$ polypeptide expressing cell.

[0201] This invention is directed to a pharmaceutical composition which comprises an amount of an above-identified antibody effective to block binding of a ligand to the $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

[0202] This invention is directed to a transgenic, nonhuman mammal expressing a $GABA_BR1/R2$ receptor, which is not naturally expressed by the mammal.

[0203] This invention is directed to a transgenic, nonhuman mammal comprising a homologous recombination knockout of the native $GABA_BR1/R2$ receptor.

[0204] In one embodiment, the transgenic nonhuman mammal is a mouse.

[0205] This invention is directed to a method of detecting the presence of a $GABA_BR1/R2$ receptor on the surface of a cell which comprises contacting the cell with an above-identified antibody under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a $GABA_BR1/R2$ receptor on the surface of the cell.

[0206] This invention is directed to a method of determining the physiological effects of varying levels of activity of $GABA_BR1/R2$ receptors which comprises producing an above-identified transgenic nonhuman mammal whose levels of $GABA_BR1/R2$ receptor activity vary due to the presence of an inducible promoter which regulates $GABA_BR1/R2$ receptor expression.

[0207] This invention is directed to a method of determining the physiological effects of varying levels of activity of $GABA_BR1/R2$ receptors which comprises producing a panel of above-identified transgenic nonhuman mammals, each expressing a different amount of $GABA_BR1/R2$ receptor.

[0208] This invention is directed to a method for identifying an antagonist capable of alleviating an abnormality, by decreasing the activity of a $GABA_BR1/R2$ receptor comprising administering a compound to a above-identified transgenic nonhuman mammal, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as the antagonist.

[0209] This invention is directed to an antagonist identified by an above-identified method.

[0210] This invention is directed to a pharmaceutical composition comprising an above-identified antagonist and a pharmaceutically acceptable carrier.

[0211] This invention is directed to a method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a $GABA_BR1/R2$ receptor which comprises administering to a subject an effective amount of an above-identified pharmaceutical composition, thereby treating the abnormality.

[0212] This invention is directed to a method for identifying an agonist capable of alleviating an abnormality, by increasing the activity of a $GABA_BR1/R2$ receptor comprising administering a compound to an above-identified transgenic nonhuman mammal, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as the agonist.

[0213] This invention is directed to an agonist identified by an above-identified method.

[0214] This invention is directed to a pharmaceutical composition comprising an above-identified agonist and a pharmaceutically acceptable carrier.

[0215] This invention is directed to a method for treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a $GABA_BR1/R2$ receptor which comprises administering to a subject an effective amount of an above-identified pharmaceutical composition, thereby treating the abnormality.

[0216] This invention is directed to a cell which expresses on its surface a mammalian $GABA_BR1/R2$ receptor that is not naturally expressed on the surface of such cell.

[0217] This invention is directed to a cell, wherein the mammalian $GABA_BR1/R2$ receptor comprises two polypeptides, one of which is a $GABA_BR2$ polypeptide and another of which is a $GABA_BR1$ polypeptide.

[0218] This invention is directed to a process for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor.

[0219] This invention is directed to a process for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises contacting a membrane fraction from a cell extract of cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor.

[0220] In one embodiment, the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

[0221] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

[0222] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same sequence as the amino acid sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0223] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the amino acid sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0224] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

[0225] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0226] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0227] In another embodiment, the compound is not previously known to bind to a $GABA_BR1/R2$ receptor.

[0228] This invention is directed to a compound identified by an above-identified process.

[0229] In one embodiment, the cell is an insect cell.

[0230] In another embodiment, the cell is a mammalian cell.

[0231] In another embodiment, the cell is nonneuronal in origin.

[0232] In another embodiment, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk−) cell.

[0233] In another embodiment, the compound is not previously known to bind to a $GABA_BR1/R2$ receptor.

[0234] This invention is directed to a compound identified by an above-identified process.

[0235] This invention is directed to a process involving competitive binding for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises separately contacting cells expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor, a decrease in the binding of the second chemical compound to the $GABA_BR1/R2$ receptor in the presence of the chemical compound indicating that the chemical compound binds to the $GABA_BR1/R2$ receptor.

[0236] This invention is directed to a process involving competitive binding for identifying a chemical compound which specifically binds to a human $GABA_BR1/R2$ receptor which comprises separately contacting a membrane fraction from a cell extract of cells expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor, a decrease in the binding of the second chemical compound to the $GABA_BR1/R2$ receptor in the presence of the chemical compound indicating that the chemical compound binds to the $GABA_BR1/R2$ receptor.

[0237] In one embodiment, the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

[0238] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by plasmid BO-55 (ATCC Accession No. 209104).

[0239] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 23A-23D (SEQ ID NO:47).

[0240] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the amino acid sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0241] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

[0242] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0243] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0244] In another embodiment, the cell is an insect cell.

[0245] In another embodiment, the cell is a mammalian cell.

[0246] In another embodiment, the cell is nonneuronal in origin.

[0247] In another embodiment, the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk−) cell.

[0248] In another embodiment, the compound is not previously known to bind to a $GABA_BR1/R2$ receptor.

[0249] This invention is directed to a compound identified by an above-identified process.

[0250] This invention is directed to a method of screening a plurality of chemical compounds not known to bind to a $GABA_BR1/R2$ receptor to identify a compound which specifically binds to the $GABA_BR1/R2$ receptor, which comprises

[0251] (a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with a compound known to bind specifically to the $GABA_BR1/R2$ receptor;

[0252] (b) contacting the same cells as in step (a) with the plurality of compounds not known to bind specifically to the $GABA_BR1/R2$ receptor, under conditions permitting binding of compounds known to bind the $GABA_BR1/R2$ receptor;

[0253] (c) determining whether the binding of the compound known to bind specifically to the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of the compounds, relative to the binding of the compound in the absence of the plurality of compounds, and if the binding is reduced;

[0254] (d) separately determining the extent of binding to the $GABA_BR1/R2$ receptor of each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the $GABA_BR1/R2$ receptor.

[0255] This invention is directed to a method of screening a plurality of chemical compounds not known to bind to a $GABA_BR$ 1/R2 receptor to identify a compound which specifically binds to the $GABA_BR1/R2$ receptor, which comprises

[0256] (a) contacting a membrane fraction extract from cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with a compound known to bind specifically to the $GABA_BR1/R2$ is receptor;

[0257] (b) contacting the same membrane fraction as in step (a) with the plurality of compounds not known to bind specifically to the $GABA_BR1/R2$ receptor, under conditions permitting binding of compounds known to bind the $GABA_BR1/R2$ receptor;

[0258] (c) determining whether the binding of the compound known to bind specifically to the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds, and if the binding is reduced;

[0259] (d) separately determining the extent of binding to the $GABA_BR1/R2$ receptor of each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the $GABA_BR1/R2$ receptor.

[0260] In one embodiment, the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

[0261] In one embodiment, the cell is a mammalian cell.

[0262] In one embodiment, the mammalian cell is non-neuronal in origin.

[0263] In one embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk–) cell, a CHO cell, a mouse Y1 cell or an NIH-3T3 cell.

[0264] This invention is directed to a process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor agonist which comprises contacting cells with the compound under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting an increase in $GABA_BR1/R2$ receptor activity, so as to thereby determine whether the compound is a $GABA_BR1/R2$ receptor agonist.

[0265] This invention is directed to a process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor antagonist which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the compound in the presence of a known $GABA_BR1/R2$ receptor agonist, under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting a decrease in $GABA_BR1/R2$ receptor activity, so as to thereby determine whether the compound is a $GABA_BR1/R2$ receptor antagonist.

[0266] Expression of genes in Xenopus oocytes is well known in the art (A. Colc*/man, *Transcription and Translation: A Practical Approach* (B. D. Hanes, S. J. Higgins, eds., pp 271-302, IRL Press, Oxford, 1984; Y. Masu et al., *Nature* 329:21583-21586, 1994) and is performed using microinjection of native mRNA or in vitro synthesized mRNA into frog oocytes. The preparation of in vitro synthesized mRNA can be performed by various standard techniques (J. Sambrook et al., *Molecular Cloning: A Laboratory Manual,* Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1989) including using T7 polymerase with the mCAP RNA capping kit (Stratagene).

[0267] In one embodiment, the cells additionally express nucleic acid encoding GIRK1 and GIRK4.

[0268] In another embodiment, the $GABA_BR2$ receptor is a mammalian $GABA_BR2$ receptor.

[0269] This invention is directed to a pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor agonist determined to be an agonist by an above-identified process effective to increase activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

[0270] This invention is directed to a pharmaceutical, wherein the $GABA_BR1/R2$ receptor agonist was not previously known.

[0271] This invention is directed to a pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor antagonist determined to be an antagonist an

above-identified process effective to reduce activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

[0272] This invention is directed to a pharmaceutical composition, wherein the $GABA_BR1/R2$ receptor antagonist was not previously known.

[0273] This invention is directed to a process for determining whether a chemical compound activates a $GABA_BR1/R2$ receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the chemical compound under conditions suitable for activation of the $GABA_BR1/R2$ receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the $GABA_BR1/R2$ receptor.

[0274] In one embodiment, the second messenger response comprises potassium channel activation and the change in second messenger is an increase in the level of potassium current.

[0275] This invention is directed to a process for determining whether a chemical compound inhibits activation of a $GABA_BR1/R2$ receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical compound known to activate the $GABA_BR1/R2$ receptor, and with only the second chemical compound, under conditions suitable for activation of the $GABA_BR1/R2$ receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the $GABA_BR1/R2$ receptor.

[0276] In one embodiment, the second messenger response comprises potassium channel activation and the change in second messenger response is a smaller increase in the level of inward potassium current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

[0277] This invention is directed to an above-identified process, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

[0278] In one embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

[0279] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 4A-4D (SEQ ID NO:4).

[0280] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 23A-23D (SEQ ID NO:47).

[0281] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence, shown in FIGS. 23A-23D (SEQ ID NO:47).

[0282] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

[0283] This invention is directed to an above-identified process, wherein the cell is an insect cell.

[0284] This invention is directed to an above-identified process, wherein the cell is a mammalian cell.

[0285] In one embodiment, the mammalian cell is non-neuronal in origin.

[0286] In another embodiment, the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk-) cell.

[0287] In another embodiment, the compound was not previously known to activate or inhibit a $GABA_BR1/R2$ receptor.

[0288] This invention is directed to a compound determined by an above-identified process.

[0289] This invention is directed to a pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor agonist determined by an above-identified process effective to increase activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

[0290] In one embodiment, the $GABA_BR1/R2$ receptor agonist was not previously known.

[0291] This invention is directed to a pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor antagonist determined by an above-identified process effective to reduce activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

[0292] In one embodiment, the $GABA_BR1/R2$ receptor antagonist was not previously known.

[0293] This invention is directed to method of screening a plurality of chemical compounds not known to activate a $GABA_BR1/R2$ receptor to identify a compound which activates the $GABA_BR1/R2$ receptor which comprises:

[0294] (a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the plurality of compounds not known to activate the $GABA_BR1/R2$ receptor, under conditions permitting activation of the $GABA_BR1/R2$ receptor;

[0295] (b) determining whether the activity of the $GABA_BR1/R2$ receptor is increased in the presence of the compounds, and if it is increased;

[0296] (c) separately determining whether the activation of the $GABA_BR1/R2$ receptor is increased by

each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which activates the $GABA_BR1/R2$ receptor.

[0297] In one embodiment, the cells express nucleic acid encoding GIRK1 and GIRK4.

[0298] In another embodiment, the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

[0299] This invention is directed to a method of screening a plurality of chemical compounds not known to inhibit the activation of a $GABA_BR1/R2$ receptor to identify a compound which inhibits the activation of the $GABA_BR1/R2$ receptor, which comprises:

[0300] (a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the plurality of compounds in the presence of a known $GABA_BR1/R2$ receptor agonist, under conditions permitting activation of the $GABA_BR1/R2$ receptor;

[0301] (b) determining whether the activation of the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of compounds, relative to the activation of the $GABA_BR1/R2$ receptor in the absence of the plurality of compounds, and if it is reduced;

[0302] (c) separately determining the inhibition of activation of the $GABA_BR1/R2$ receptor for each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such a plurality of compounds which inhibits the activation of the $GABA_BR1/R2$ receptor.

[0303] In one embodiment, the cells express nucleic acid encoding GIRK1 and GIRK4.

[0304] In one embodiment, the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

[0305] In another embodiment, wherein the cell is a mammalian cell.

[0306] In another embodiment, the mammalian cell is non-neuronal in origin.

[0307] In another embodiment, the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk–) cell or an NIH-3T3 cell.

[0308] This invention is directed to a pharmaceutical composition comprising a compound identified by an above-identified method, effective to increase $GABA_BR1/R2$ receptor activity and a pharmaceutically acceptable carrier.

[0309] This invention is directed to a pharmaceutical composition comprising a compound identified by an above-identified method, effective to decrease $GABA_BR1/R2$ receptor activity and a pharmaceutically acceptable carrier.

[0310] This invention is directed to a process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor agonist, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, separately contacting the membrane fraction with both the chemical compound and GTPYS, and with only GTPYS, under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting GTPYS binding to the membrane fraction, an increase in GTPYS binding in the presence of the compound indicating that the chemical compound activates the $GABA_BR1/R2$ receptor.

[0311] This invention is directed to a process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor antagonist, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, separately contacting the membrane fraction with the chemical compound, GTPYS and a second chemical compound known to activate the $GABA_BR1/R2$ receptor, with GTPYS and only the second compound, and with GTPYS alone, under conditions permitting the activation of the $GABA_BR1/R2$ receptor, detecting GTPYS binding to each membrane fraction, and comparing the increase in GTPYS binding in the presence of the compound and the second compound relative to the binding of GTPYS alone, to the increase in GTPYS binding in the presence of the second chemical compound known to activate the $GABA_BR1/R2$ receptor relative to the binding of GTPYS alone, a smaller increase in GTPYS binding in the presence of the compound and the second compound indicating that the compound is a $GABA_BR1/R2$ receptor antagonist.

[0312] In one embodiment, the $GABA_BR2$ receptor is a mammalian $GABA_BR2$ receptor.

[0313] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

[0314] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 4A-4D (SEQ ID NO:4).

[0315] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

[0316] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 23A-23D (SEQ ID NO:47).

[0317] In another embodiment, the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

[0318] In another embodiment, the cell is an insect cell.

[0319] In another embodiment, the cell is a mammalian cell.

[0320] In another embodiment, the mammalian cell is nonneuronal in origin.

[0321] In another embodiment, the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk−) cell.

[0322] In another embodiment, the compound was not previously known to be an agonist or antagonist of a $GABA_BR1/R2$ receptor.

[0330] This invention directed to a method of treating Alzheimer's disease in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor antagonist effective to treat Alzheimer's disease in the subject.

[0331] This invention is directed to a peptide selected from the group consisting of:

a) PLYSILSALTILGMIMASAFLFFNIK N; (SEQ ID NO:48)

b) LIILGGMLSYASIFLFGLDGSFVSEK T; (SEQ ID NO:49)

c) CTVRTWILTVGYTTAFGAMFAKTWR; (SEQ ID NO:50)

d) QKLLVIVGGMLLIDLCLLICWQ; (SEQ ID NO:51)

e) MTIWLGIVYAYKGLLMLFGCFLAW; (SEQ ID NO:52)

f) ALNDSKYIGMSVYNVGIMCIIGAAV; (SEQ ID NO:53) and

g) CIVALVIIFCSTITLCLVFVPKLITLRTN. (SEQ ID NO:54)

[0323] This invention is directed to a compound determined to be an agonist or antagonist of a $GABA_BR1/R2$ receptor by an above-identified process.

[0324] This invention is directed to a method of treating spasticity in a subject which comprises administering to the subject an amount of a compound which is an agonist of a $GABA_BR1/R2$ receptor effective to treat spasticity in the subject.

[0325] This invention is directed to a method of treating asthma in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to treat asthma in the subject.

[0326] This invention is directed to a method of treating incontinence in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to treat incontinence in the subject.

[0327] This invention is directed to method of decreasing nociception in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to decrease nociception in the subject.

[0328] This invention is directed to a use of a $GABA_BR2$ agonist as an antitussive agent which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective as an antitussive agent in the subject.

[0329] This invention is directed to a method of treating drug addiction in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to treat drug addiction in the subject.

[0332] This invention is directed to a compound that prevents the formation of a $GABA_BR1/R2$ receptor complex.

[0333] Transmembrane peptides derived from $GABA_BR2$ sequences may modulate the functional activity of $GABA_BR1/R2$ receptors. One mode of action involves the destruction of the $GABA_BR1/R2$ receptor complex via competitive displacement of the $GABA_BR2$ polypeptide subunit by the peptide upon binding to the $GABA_BR1$ polypeptide subunit. The peptides may be synthesized using standard solid phase F-moc peptide synthesis protocol using an Advanced Chemtech 396 Automated Peptide Synthesizer.

[0334] Additional $GABA_B$ subtypes in hypothalamus and caudate putamen are predicted due to the under-representation of $GABA_BR2$ hybridization signals. These novel $GABA_B$ proteins and others may be identified by using $GABA_BR2$ polypeptides in co-immunoprecipitation experiments.

[0335] This invention provides a process for making a composition of matter which specifically binds to a $GABA_BR1/R2$ receptor which comprises identifying a chemical compound using any of the processes described herein for identifying a compound which binds to and/or activates or inhibits activation of a $GABA_BR1/R2$ receptor and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof. In one embodiment, the $GABA_BR1/R2$ receptor is a human $GABA_BR1/R2$ receptor.

[0336] This invention further provides a process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by any of the processes described herein for identifying a compound which binds to and/or activates or inhibits activation of a $GABA_BR1/R2$ receptor or a novel

structural and functional analog or homolog thereof. In one embodiment, the $GABA_BR1/R2$ receptor is a human $GABA_BR1/R2$ receptor.

[0337] Thus, once the gene for a targeted receptor subtype is cloned, it is placed into a recipient cell which then expresses the targeted receptor subtype on its surface. This cell, which expresses a single population of the targeted human receptor subtype, is then propagated resulting in the establishment of a cell line. This cell line, which constitutes a drug discovery system, is used in two different types of assays: binding assays and functional assays. In binding assays, the affinity of a compound for both the receptor subtype that is the target of a particular drug discovery program and other receptor subtypes that could be associated with side effects are measured. These measurements enable one to predict the potency of a compound, as well as the degree of selectivity that the compound has for the targeted receptor subtype over other receptor subtypes. The data obtained from binding assays also enable chemists to design compounds toward or away from one or more of the relevant subtypes, as appropriate, for optimal therapeutic efficacy. In functional assays, the nature of the response of the receptor subtype to the compound is determined. Data from the functional assays show whether the compound is acting to inhibit or enhance the activity of the receptor subtype, thus enabling pharmacologists to evaluate compounds rapidly at their ultimate human receptor subtypes targets permitting chemists to rationally design drugs that will be more effective and have fewer or substantially less severe side effects than existing drugs.

[0338] Approaches to designing and synthesizing receptor subtype-selective compounds are well known and include traditional medicinal chemistry and the newer technology of combinatorial chemistry, both of which are supported by computer-assisted molecular modeling. With such approaches, chemists and pharmacologists use their knowledge of the structures of the targeted receptor subtype and compounds determined to bind and/or activate or inhibit activation of the receptor subtype to design and synthesize structures that will have activity at these receptor subtypes.

[0339] Combinatorial chemistry involves automated synthesis of a variety of novel compounds by assembling them using different combinations of chemical building blocks. The use of combinatorial chemistry greatly accelerates the process of generating compounds. The resulting arrays of compounds are called libraries and are used to screen for compounds (lead compounds) that demonstrate a sufficient level of activity at receptors of interest. Using combinatorial chemistry it is possible to synthesize focused libraries of compounds anticipated to be highly biased toward the receptor target of interest.

[0340] Once lead compounds are identified, whether through the use of combinatorial chemistry or traditional medicinal chemistry or otherwise, a variety of homologs and analogs are prepared to facilitate an understanding of the relationship between chemical structure and biological or functional activity. These studies define structure activity relationships which are then used to design drugs with improved potency, selectivity and pharmacokinetic properties. Combinatorial chemistry is also used to rapidly generate a variety of structures for lead optimization. Traditional medicinal chemistry, which involves the synthesis of compounds one at a time, is also used for further refinement and to generate compounds not accessible by automated techniques. Once such drugs are defined the production is scaled up using standard chemical manufacturing methodologies utilized throughout the pharmaceutical and chemistry industry.

[0341] This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

Materials and Methods

DNA Sequencing

[0342] DNA sequences were determined using an ABI PRISM 377 DNA Sequencer (Perkin-Elmer, Foster City, Calif.) according to the manufacturer's instructions.

Hybridization Methodology

[0343] Probes were end-labeled with polynucleotide kinase according to the manufacturer's instructions (Boehringer-Mannheim). Hybridization was performed on Zeta-Probe membrane (Bio-Rad, CA) at reduced stringency: 40° C. in a solution containing 25% formamide, 5×SSC (1×SSC=0.15 M NaCl, 0.015 M sodium citrate), 1×Denhardt's solution (0.02% polyvinylpyrrolindone, 0.02% Ficoll, 0.02% bovine serum albumin) and 25 μg/μL sonicated salmon sperm DNA. Membrane strips were washed at 40° C. in 0.1×SSC containing 0.1% SDS and exposed at −70° C. to Kodak XAR film in the presence of an intensifying screen.

[0344] The nucleotide sequences of the hybridization probes are shown below:

T-891: 5'-AGGGATGCTTTCCTATGCTTCCATATTTCTCTTTGGCCTTGATGG-3' (SEQ ID NO:5)
Nucleotides 1449–1493 of TL-267, forward strand.

T-892: 5'-CAATGTGCAGTTCTGCATCGTGGCTCTGGTCATCATCTTCTGCAG-3' (SEQ ID NO:6)
Nucleotides 2022–2066 of TL-267, forward strand.

PCR Methodology

[0345] PCR reactions were carried out using a PE 9600 (Perkin-Elmer) PCR cycler in 20 μL volumes using Expand Long Template Polymerase (Boehringer-Mannheim) and the manufacturer's buffer 1 for internal PCR primers or manufacturer's buffer 2 for vector-anchored PCR. Reactions were run using a program consisting of 35 cycles of 94° C. for 30 sec., 68° C. for 20 sec, and 72° C. for 1 min, with a pre-incubation at 95° C. for 5 min and post-incubation hold at 4° C.

[0346] Nucleotide sequences of the primer sets used in PCR reactions are shown below:

T-94: 5'-CTTCTAGGCCTGTACGGAAGTGTT-3' (SEQ ID NO: 7); vector, forward primer.

T-95: 5'-GTTGTGGTTTGTCCAAACTCATCAAT-3' (SEQ ID NO: 8); vector, reverse primer.

T-887: 5'-GGGATGAGTGTCTACAACGTGGGG-3' (SEQ ID NO: 9); nucleotides 1948–1971 of TL-267, forward primer.

T-888: 5'-TGCGTTGCTGCATCTGGGTTTGTTCT-3' (SEQ ID NO: 10); nucleotides 2138–2113 of TL-267, reverse primer.

T-889: 5'-ATCTCCCTACCTCTCTACAGCATCCT-3' (SEQ ID NO: 11); nucleotides 1300–1325 of TL-267, forward primer.

T-890: 5'-CAGGTCCTGACGGTGCAAAGTGTTTC-3' (SEQ ID NO: 12); nucleotides 1544–1519 of TL-267, reverse primer.

T-921: 5'-TGACGCAAGACGTTCAGAGGTTCTCT-3' (SEQ ID NO: 13); nucleotides 473–498 of TL-267, forward primer.

T-922: 5'-TGTAGCCTTCCATGGCAGCAAGCAGA-3' (SEQ ID NO: 14); nucleotides 814–789 of TL-267, reverse primer.

T-923: 5'-AGAGAACCTCTGAACGTCTTGCGTCA-3' (SEQ ID NO: 15); nucleotides 498–473 of TL-267, reverse primer.

T-935: 5'-GGCTCTGTTGTGTTCCACTGTAGCTG-3' (SEQ ID NO: 16); nucleotides 2483–2458 of TL-267, reverse primer.

T-938: 5'-TCATGC-CGCTCAC-CAAGGAGGTG-GCC-3' (SEQ ID NO: 17); nucleotides 53 to 78 of TL-267, forward primer.

T-939: 5'-GGCCACCTCCTTGGTGAGCGGCATGA-3' (SEQ ID NO: 18); nucleotides 78 to 53 of TL-267, reverse primer.

T-947: 5'-TGAGTGAGCAGAGTCCAGAGCCGT-3' (SEQ ID NO: 19); nucleotides -68 to -45 of TL-267, forward primer.

T-948: 5'-ATGGATGGGAGGTAGGCGTGGTGGAG-3' (SEQ ID NO: 20); nucleotides 2591–2566 of TL-267, reverse primer.

Preparation of Human Hippocampal CDNA Library

[0347] Total RNA was prepared by a modification of the guanidine thiocyanate method, from 6 grams of human hippocampus. Poly A+RNA was purified with a FastTrack kit (Invitrogen Corp., San Diego, Calif.). Double stranded (ds) cDNA was synthesized from 4 μg of poly A+ RNA according to Gübler and Hoffman (1983), except that ligase was omitted in the second strand cDNA synthesis. The resulting DS cDNA was ligated to BstxI/EcoRI adaptors (Invitrogen Corp.), the excess of adaptors was removed by exclusion chromatography. High molecular weight fractions were ligated in pcEXV.BS (An Okayama and Berg expression vector) cut by BstxI as described by Aruffo and Seed (1987). The ligated DNA was electroporated in *E. coli* MC 1061 (Gene Pulser, Biorad). A total of 2.2×10^6 independent clones with an insert mean size of approximately 3 kb was generated. The library was plated on Petri dishes (Ampicillin selection) in pools of 0.4 to 1.2×10^4 independent clones. After 18 hours amplification, the bacteria from each pool were scraped, resuspended in 4 mL of LB media and 1.5 mL processed for plasmid purification by the alkali method (Sambrook et al, 1989). 1 mL aliquots of each bacterial pool were stored at -85° C. in 20% glycerol.

BLAST Search that Identified a Novel 7-TM Protein Sequence

[0348] Sequence analysis was performed with the Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, Wis. The rat $GABA_BR1a$ amino acid sequence (Kaupmann et al. (1997) Nature 386:239) was used as a query to search the EST division of GenBank with BLAST. Two entries, T07621 and Z43654, had probability scores that suggested significant amino acid homology to the $GABA_BR1a$ polypeptide. T07621 had sequence homology from the beginning of the first transmembrane domain to the beginning of third transmembrane domain of the $GABA_BR1a$ polypeptide. Z43654 had sequence homology from the sixth transmembrane domain to the seventh transmembrane domain of the $GABA_BR1a$ polypeptide. The sequence documentation for T07621 and Z43654 was retrieved with Entrez (NCBI) and neither sequence was annotated as having homology to any 7-TM spanning protein.

T07621 and Z43654 are Part of the Same Sequence

[0349] A series of PCR reactions were carried out on human hippocampus DNA with multiple primer sets: primer set T-887/T-888 designed to Z43654 sequence; primer set T-889/T-890 designed to the T07621 sequence; and primer set T-889/T-888 designed to the forward strand of T07621 and the reverse stand of Z43654. The PCR products was loaded on duplicate lanes of an agarose gel and the DNA was southern blotted to a Zeta-Probe membrane (Bio-Rad, CA). The regions of the membrane corresponding to the individual lanes on the gel were cut to produce membrane strips that contained duplicate samples of the DNA. One set of membrane strips was hybridized with T-891, a probe specific for the T07621 sequence. Another set of membranes was hybridized with T-892, a probe specific to the Z43654 sequence. The membrane from primer set T-887/T-888 hybridized with probe T-892 for the Z43654 sequence. The membrane from primer set T-889/T-890 hybridized with probe T-891 for the T07621 sequence. The membrane from primer set T889/T-888 hybridized with both the T-891 and T-892 probes.

Isolating the Full-length Human cDNA by PCR Sib Selection

[0350] PCR reactions were carried out on bacterial pools containing a human hippocampus cDNA library. Primer set T-888/T-889 was used to identify the bacterial pools that contained a portion of the novel receptor. Vector-anchored PCR was carried out on the positive pools to determine which pool contained the longest cDNA insert. Four primer sets were used for the vector-anchored PCR: T-94/T-888, T-94/T889, T-95/T888, and T-95/T889. Pool 365 was identified having the longest cDNA inset and the plasmid was sib selected (McCormick, 1987). The nucleotide sequence of

clone 365-9-7-4, designated TL-260, was translated into amino acids and compared to the amino acid sequence of the rat $GABA_BR1a$ polypeptide. Relative the rat $GABA_BR$ 1a amino acid sequence, TL-260 was truncated at the amino terminus.

[0351] A set of PCR primers (T-921/T-922) was made to the 5' region of TL-260 and was used to re-screen the bacterial pools of the human hippocampus library for the missing segment of the novel clone. Vector-anchored PCR was carried out on the positive pools to determine which pool contained the longest cDNA insert. Four primer sets were used for the vector-anchored PCR: T-94/T-921, T-94/T922, T-95/T921, and T-95/T-922. Pool 299 contained the most 5' sequence. A PCR product derived from the primer set T-94/T-923 was isolated (T-261) and sequenced. The putative amino acids derived from TL-261 were compared to the rat $GABA_BR1$ sequence. TL-261 contained an initiation codon but didn't contain a stop codon upstream of the initiation codon.

[0352] A set of PCR primers (T-938/T-935) was made to the 5'region of TL-261 and was used to re-screen the bacterial pools of the human hippocampus library for additional sequence. Vector-anchored PCR was carried out on the positive pools to determine which pool contained the longest cDNA insert. Four primer sets were used for the vector-anchored PCR: T-94/T-938, T-94/T939, T-95/T938, and T-95/T-939. A PCR product derived from primer set T-95/T-939 was isolated (T-261a) and sequenced. The putative amino acids derived from T-261a were compared to the rat GABA-1 amino acid sequence. T-261a contained an initiation codon and an in-frame upstream stop codon.

[0353] From the vector-anchored PCR, pool 389 contained the longest cDNA insert. This pool was sib selected with the primer set T-947/T-935. The resulting plasmid, 389-20-29-2, was designated TL-266 and was sequenced.

Construction of $GABA_BR2$ Polypeptide in Expression Vector

[0354] A Cla-I-Xba-I fragment from TL-266 was subcloned into the expression vector pEXJ.HRT3T7 and designated TL-267. This plasmid (TL-267) was deposited on Jun. 10, 1997, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md. 20852, U.S.A. under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure and was accorded ATCC Accession No. 209103.

Generation of Rat $GABA_BR2$ PCR Product

[0355] cDNA from rat hippocampus and rat cerebellum were amplified in 50 μL PCR reaction mixtures using the Expand Long Template PCR System (as supplied and described by the manufacturer, Boehringer Mannheim) using a program consisting of 40 cycles of 94° C. for 1 min, 50° C. for 2 min, and 68° C. for 2 min, with a pre- and post-incubation of 95° C. for 5 min and 68° C. for 7 min, respectively. PCR primers for rat $GABA_BR2$ were designed against the human $GABA_BR2$ sequence: BB 257, forward primer in the first transmembrane domain, and BB 258, reverse primer in the seventh transmembrane domain. The single 780 bp fragment from both rat hippocampus and rat cerebellum were isolated from a 1% agarose gel, purified using a GENECLEAN III kit (BIO 101, Vista, Calif.) and sequenced using AmpliTaq DNA Polymerase, FS (Perkin Elmer). The sequence was run on an ABI PRISM 377 DNA Sequencer and analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, Wis.). This sequence was used to design PCR primers for the rat $GABA_BR2$ gene.

Construction and Screening of a Rat Hypothalamic cDNA Library

[0356] Poly A^+ RNA was purified from rat hypothalamic RNA (Clontech) using a FastTrack kit (Invitrogen, Corp.). DS-cDNA was synthesized from 5 μg of poly A+ RNA according to Gubler and Hoffman (1983) with minor modifications. The resulting cDNA was ligated to BstXI adaptors (Invitrogen, Corp.) And the excess adapters removed by exclusion column chromatography. High molecular weight fractions of size-selected ds-cDNA were ligated in pEXJ.T7, an Okayama and Berg expression vector modified from pcEXV (Miller and Germain, 1986) to contain BstXI, other additional restriction sites, and a T7 promoter. A total of 100,000 independent clones with a mean insert size of 3.7 kb were generated. The library was amplified on agar plates (Ampicillin selection) in 48 primary pools. Glycerol stocks of the primary pools screened for a rat $GABA_BR2$ gene by PCR using BB265, a forward primer from the loop between transmembrane domains 3 and 4 from the sequence determined above and BB266, a reverse primer from the sixth transmembrane domain from the sequence determined above. The conditions for PCR were 1 min at 94° C., 4 min at 68° C. for 40 cycles, with a pre- and post-incubation of 5 min at 95° C. and 7 min at 68° C., respectively. To determine which pools had the largest inserts, positive pools were screened by PCR using the vector primers BB172 or BB173, and a gene-specific primer BB265 or BB266. One positive primary pool, I-47, was subdivided into 24 pools of 1000 clones, and grown in LB medium overnight. Two μL of cultures were screened by PCR using primers BB172 and BB266. One positive subpool, I-47-4 was subdivided into 10 pools of 200 clones and plated on agar plates (ampicillin selection). Colonies were transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, N.H.), denatured in 0.4 N NaOH, 1.5 M NaCl, renatured in 1M Tris, 1.5 M NaCl, and UV cross-linked. Filters were hybridized overnight at 40° C. in a buffer containing 50 % formamide, 0.12 M Na_2HPO_4 (pH7.2), 0.25M NaCl, 7%SDS, 25 mg/L ssDNA and 10^6 cpm/mL of a cDNA probe corresponding to transmembrane domains 1 to 7 of rat $GABA_BR2$, labeled with [^{32}P]dCTP (3000 Ci/mmol, NEN) using a random prime labeling kit (Boehringer Mannheim). Filters were washed 1x5 min then 2x20 min at room temperature in 2xSSC, 0.1% SDS then 3x20 min at 500 in 0.1xSSC, 0.1% SDS and exposed to Biomax MS film (Kodak) for 3 hours. Four closely clustering colonies which appeared to hybridize were re-screened individually by PCR using primers BB265 and BB266, primers BB265 and BB55, primers BB265 and BB56, and primers BB266 and BB55. The conditions for PCR were 30 sec at 94° C., 2.5 min at 68° C. for 32 cycles, with a pre- and post-incubation of 5 min at 95° C. and 5 min at 68° C. respectively. One positive colony, I-47-4-2, was amplified overnight in 10 mL TB media and processed for

plasmid purification using a standard alkaline lysis miniprep procedure followed by a PEG precipitation. This plasmid was designated BO54 and partially sequenced using AmpliTaq DNA Polymerase, FS (Perkin Elmer). The sequence was run on an ABI PRISM 377 DNA Sequencer and analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, Wis.). BO54 was in the wrong orientation for expression in mammalian cells. To obtain a clone in the correct orientation, an EcoRI restriction fragment from BO54 was subcloned into the vector pEXJ. Transformants were screened by PCR using the primers BB56 and BB268 under the following conditions: 30 sec at 94° C., 2.5 min at 68° C. for 32 cycles, with a pre- and post-incubation of 5 min at 95° C. and 3 min at 68° C. respectively. One transformant in the correct orientation was amplified overnight in 100 ml

[0357] TB media and processed for plasmid purification using a standard alkaline lysis miniprep procedure followed by a PEG precipitation. This plasmid was designated BO55 and sequenced using AmpliTaq DNA Polymerase, FS (Perkin Elmer). Plasmid BO-55 was deposited with the ATCC on Jun. 10, 1997, and was accorded ATCC Accession No. 209104. The sequence of BO-55 was determined using an ABI PRISM 377 DNA Sequencer and analyzed using the Wisconsin Package (GCG, Genetics Computer Group, Madison, Wis.).

Primers Used

[0358]

BB257: 5'-CTCTCTGCCCTCACCATCCTCGGGAT-3' (SEQ ID NO:21)

BB258: 5'-GACTCCGGCTCGAATACCAGGCAGAG-3' (SEQ ID NO:22)

BB265: 5'-CCATGTTTGCAAAGACCTGGAGGGTCC-3' (SEQ ID NO:23)

BB266: 5'-GGTCACGCGTCAGGAAAGAGACAGCAG-3' (SEQ ID NO:24)

BB172: 5'-AAGCTTCTAGAGATCCCTCGACCTC-3' (SEQ ID NO:25)

BB173: 5'-AGGCGCAGAACTGGTAGGTATGGAA-3' (SEQ ID NO:26)

BB55: 5'-CTTCTAGGCCTGTACGGAAGTGTTA-3' (SEQ ID NO:27)

BB56: 5'-GTTGTGGTTTGTCCAAACTCATCAATG-3' (SEQ ID NO:28)

BB268: 5'-CTGCTGTCTCTTTCCTGACGCGTGACC-3' (SEQ ID NO:29).

Generation of DNA Coding for Rat $GABA_B$1b and $GABA_B$1a Polylpeptides

[0359] The gene encoding the rat $GABA_B$R1b polypeptide was obtained by screening the same rat hypothalamic library used for $GABA_B$R2 with primers based on the original publication of the clone by Kaupmann, et al., 1997. A partial clone lacking the first 55 nucleotides was identified and ligated to a PCR fragment containing the missing base pairs to obtain the full length clone. A restriction fragment containing the entire coding region of $GABA_B$R1b was subcloned into the mammalian expression vector pEXJ.T7 and designated "BO58". A rat $GABA_B$1a polypeptide clone was obtained by ligating a restriction fragment of the $GABA_B$1b clone, which contained the common region of the $GABA_B$1 gene, to a PCR product containing the $GABA_B$1a-specific 5' end.

In Situ Hybridization Experiments for $GABA_B$R2 mRNA Animals

[0360] Male Sprague-Dawley rats (Charles Rivers, Rochester, N.Y.) were euthanized using CO_2, decapitated, and their brains immediately removed and rapidly frozen on crushed dry ice. Coronal sections of brain tissue were cut at 11 μm using a cryostat and thaw-mounted onto poly-L-lysine-coated slides and stored at −20° C. until use.

Tissue Preparation

[0361] Prior to hybridization, the tissues were fixed in 4% paraformaldehyde/PBS pH 7.4 followed by two washes in PBS (Specialty Media, Lavallette, N.J.). Tissues were then treated in 5 mM dithiothreitol, rinsed in DEPC-treated PBS, acetylated in 0.1 M triethanolamine containing 0.25% acetic anhydride, rinsed twice in 2×SSC, delipidated with chloroform then dehydrated through a series of graded alcohols. All reagents were purchased from Sigma (St. Louis, Mo.).

Radioactive In Situ Hybridization Histochemistry

[0362] Oligonucleotide probes, MJ79/80, corresponding to nucleotides 354-398 and MJ109/110, corresponding to nucleotides 952-991 of the rat $GABA_B$R2 cDNA, MJ94/95, corresponding to nucleotides 151-193 of the human $GABA_B$R1a cDNA, and MJ83/84, corresponding to nucleotides 34-71 of the rat $GABA_B$R1b cDNA were used to characterize the distribution of each polypeptides's respective mRNA. The oligonucleotides were synthesized using an Expedite Nucleic Acid Synthesis System (PerSeptive Biosystems, Framingham, Mass.) and purified using 12% polyacrylamide gel electrophoresis. Additionally, sense and antisense oligonucleotides corresponding to positions 1076-1120 of $GABA_B$R1b (1424-1468 of $GABA_B$R1a) were used (BB403 and BB404).

[0363] The sequences of the oligonucleotides are:

[0364] For rat $GABA_BR2$:

Sense probe, MJ79:
5' - GCA ATA AAG TAT GGG CTG AAC CAT TTG (SEQ ID NO:36)
ATG GTG TTT GGA GGC GT - 3'

Antisense probe, MJ80:
5' - ACG CCT CCA AAC ACC ATC AAA TGG TTC (SEQ ID NO:37)
AGC CCA TAC TTT ATT GC - 3'

Sense probe, MJ109:
5' - TTT GAG CCC CTG AGC TCC AAA CAA ATC (SEQ ID NO:38)
AAG ACC ATC TCA G - 3'

Antisense probe, MJ110:
5' - CTG AGA TGG TCT TGA TTT GTT TGG AGC (SEQ ID NO:39)
TCA GGG GCT CAA A - 3'

For human GABA8R1a:
Sense probe, MJ94:
5' - AAG GCC ATC AAC TTC CTG CCT GTG GAC (SEQ ID NO:40)
TAT GAG ATC GAA TAT G - 3'

Antisense probe, MJ95:
5' - CAT ATT CGA TCT CAT AGT CCA CAG GCA (SEQ ID NO:41)
GGA AGT TGA TGG CCT T - 3'

For rat GABA8R1b:
Sense probe, MJ83:
5' - TGG CCG CTG CCT CTT CTG CTG GTG ATG (SEQ ID NO:42)
GCG GCT GGG GT - 3'
Antisense probe, MJ84:
5' - ACC CCA GCC GCC ATC ACC AGC AGA AGA (SEQ ID NO:43)
GGC AGC GGC CA - 3'
Sense probe, BB403:
5' - CCT TGG CTT TGG CCT TGA ACA AGA CGT (SEQ ID NO:44)
CTG GAG GAG GTG GTC GTT - 3'

Antisense probe, BB404:
5' - AAC GAC CAC CTC CTC CAG ACG TCT TGT (SEQ ID NO:45)
TCA AGG CCA AAG CCA AGG - 3'

[0365] Probes were 3'-end labeled with [^{35}S]dATP (1200Ci/mmol, NEN, Boston, Mass.) to a specific activity of 109 dpm/pg using terminal deoxynucleotidyl transferase (Pharmacia, Piscataway, N.J.). In situ hybridization was done with modification of the method described by Durkin, M, et al, 1995.

Nonradioactive In Situ Hybridization Histochemistry

[0366] Antisense/sense probes corresponding to nucleotides 354-398 of the rat $GABA_BR2$ cDNA, were 3'-end labeled with digoxigenin using TdT. The labeling reaction was carried out as outlined in the DIG/Genius System, (Boehringer Mannheim, Indianapolis, Ind.). Conditions used in ISHH with digoxigenin-labeled probes are the same as described above. The sections were rinsed in buffer 1, washing buffer (0.1 M Tris-HCl pH 7.5/0.15 M NaCl), pre-incubated in Blocking Solution (Buffer 1, 0.1% Triton-X and 2% normal sheep serum) for 30 minutes and then incubated for 2 hours in Blocking Solution containing anti-digoxigenin-AP Fab fragment (Boehringer Mannheim) at 1:500 dilution followed by two 10 minute washes in Buffer 1. To develop color, sections were rinsed in Detection Buffer (0.1M Tris-HCl pH 9.5/0.15M NaCl/0.05 M $MgCl_2$) for 10 minutes and then incubated overnight in Detection Buffer containing 0.5 mM NBT, 0.1 mM BCIP, and 1 mM levamisole. After color development, slides were dipped in dH2O and coverslipped using aqua mount.

[0367] Probe specificity was established by performing in situ hybridization on HEK293 cells transiently transfected with eukaryotic expression vectors containing the rat $GABA_BR1b$ and human $GABA_BR1a$ DNA or no insert for transfection. Furthermore, two pairs of hybridization probes, sense and antisense, that were targeted to different segments of the $GABA_BR2$ mRNA were used for cells and rat tissues.

Quantification

[0368] The strength of the hybridization signal obtained in various region of the rat brain was graded as weak (+), moderate (++), heavy (+++) or intense (++++). These were qualitative evaluations for each of the polypeptide mRNA distributions based on the relative optical density on the autoradiographic film and on the relative number of silver grains observed over individual cells at the microscopic level.

Cell Culture

[0369] COS-7 cells are grown on 150 mm plates in DMEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 Ag/mL streptomycin) at 37° C., 5% CO_2. Stock plates of COS-7 cells are trypsinized and split 1:6 every 3-4 days.

[0370] Human embryonic kidney 293 cells are grown on 150 mm plates in DMEM with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100

μg/mL streptomycin) at 37° C., 5% CO_2. Stock plates of 293 cells are trypsinized and split 1:6 every 3-4 days.

[0371] Mouse fibroblast LM(tk−) cells are grown on 150 mm plates in D-MEM with supplements (Dulbecco's Modified Eagle Medium with 10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 μg/mL streptomycin) at 37° C., 5% CO_2. Stock plates of LM(tk−) cells are trypsinized and split 1:10 every 3-4 days.

[0372] Chinese hamster ovary (CHO) cells are grown on 150 mm plates in HAM's F-12 medium with supplements (10% bovine calf serum, 4 mM L-glutamine and 100 units/mL penicillin/100 ug/mL streptomycin) at 37° C., 5% CO2. Stock plates of CHO cells are trypsinized and split 1:8 every 3-4 days.

[0373] Mouse embryonic fibroblast NIH-3T3 cells are grown on 150 mm plates in Dulbecco's Modified Eagle Medium (DMEM) with supplements (10% bovine calf serum, 4 mM glutamine, 100 units/mL penicillin/100 μg/mL streptomycin) at 37° C., 5% CO2. Stock plates of NIH-3T3 cells are trypsinized and split 1:15 every 3-4 days.

[0374] Sf9 and Sf21 cells are grown in monolayers on 150 mm tissue culture dishes in TMN-FH media supplemented with 10% fetal calf serum, at 27° C., no CO_2. High Five insect cells are grown on 150 mm tissue culture dishes in Ex-Cell 400™ medium supplemented with L-Glutamine, also at 27° C., no CO_2.

[0375] LM(tk−) cells stably transfected with the DNA encoding the polypeptides disclosed herein may be routinely converted from an adherent monolayer to a viable suspension. Adherent cells are harvested with trypsin at the point of confluence, resuspended in a minimal volume of complete DMEM for a cell count, and further diluted to a concentration of 10^6 cells/mL in suspension media (10% bovine calf serum, 10% 10× Medium 199 (Gibco), 9 mM $NaHCO_3$, 25 mM glucose, 2 mM L-glutamine, 100 units/mL penicillin/100 μg/mL streptomycin, and 0.05% methyl cellulose). Cell suspensions are maintained in a shaking incubator at 37° C., 5% CO_2 for 24 hours. Membranes harvested from cells grown in this manner may be stored as large, uniform batches in liquid nitrogen.

[0376] Alternatively, cells may be returned to adherent cell culture in complete DMEM by distribution into 96-well microtiter plates coated with poly-D-lysine (0.01 mg/mL) followed by incubation at 37° C., 5% CO_2 for 24 hours.

Generation of Baculovirus

[0377] The coding region of DNA encoding the polypeptides disclosed herein may be subcloned into pBlueBacIII into existing restriction sites, or sites engineered into sequences 5' and 3' to the coding region of the polypeptides. To generate baculovirus, 0.5 μg of viral DNA (BaculoGold) and 3 μg of DNA construct encoding a polypeptide may be co-transfected into 2×10^6 *Spodoptera frugiperda* insect Sf9 cells by the calcium phosphate co-precipitation method, as outlined in by Pharmingen (in "Baculovirus Expression Vector System: Procedures and Methods Manual") The cells then are incubated for 5 days at 27° C.

[0378] The supernatant of the co-transfection plate may be collected by centrifugation and the recombinant virus plaque purified. The procedure to infect cells with virus, to prepare stocks of virus and to titer the virus stocks are as described in Pharmingen's manual.

Transfection

[0379] All subtypes studied may be transiently transfected into COS-7 cells by the DEAE-dextran method, using 1 μg of DNA/10^6 cells (Cullen, 1987). In addition, Schneider 2 Drosophila cells may be cotransfected with vectors containing the gene, under control of a promoter which is active in insect cells, and a selectable resistance gene, eg., the G418 resistant neomycin gene, for expression of the polypeptides disclosed herein.

Stable Transfection

[0380] DNA encoding the polypeptides disclosed herein may be co-transfected with a G-418 resistant gene into the human embryonic kidney 293 cell line by a calcium phosphate transfection method (Cullen, 1987). Stably transfected cells are selected with G-418.

Radioligand Binding Assays

[0381] Transfected cells from culture flasks were scraped into 5 mL of Tris-HCl, 5 mM EDTA, pH 7.5, and lysed by sonication. The cell lysates were centrifuged at 1000 rpm for 5 min. at 4° C., and the supernatant was centrifuged at 30,000×g for 20 min. at 4° C. The pellet was suspended in binding buffer (50 mM Tris-HCl, 2.5 mM $CaCl_2$ at pH 7.5 supplemented with 0.1% BSA, 2 μg/mL aprotinin, 0.5 mg/mL leupeptin, and 10 μg/mL phosphoramidon). Optimal membrane suspension dilutions, defined as the protein concentration required to bind less than 10% of the added labeled compound (typically a radiolabeled compound), were added to 96-well polypropylene microtiter plates containing labeled compound, unlabeled compounds (i.e., displacing ligand in an equilibrium competition binding assay) and binding buffer to a final volume of 250 μL. In equilibrium saturation binding assays membrane preparations were incubated in the presence of increasing concentrations of labeled compound. The binding affinities of the different compounds were determined in equilibrium competition binding assays, using labeled compound, such as 1 nM [^{3}H]-CGP54626, in the presence of ten to twelve different concentrations of the displacing ligand(s). Some examples of displacing ligands included GABA, baclofen, 3APMPA, phaclofen, CGP54626, and CGP55845. Mixtures of several unlabeled test compounds (up to about 10 compounds) may also be used in competition binding assays, to determine whether one of the mixture component compounds binds to the polypeptide or receptor. Binding reaction mixtures were incubated for 1 hr at 30° C., and the reaction was stopped by filtration through GF/B filters treated with 0.5% polyethyleneimine, using a cell harvester. Where the labeled compound was a radiolabeled compound, the amount of bound compound was evaluated by gamma counting (for ^{125}I) or scintillation counting (for ^{3}H). Data were analyzed by a computerized non-linear regression program. Non-specific binding was defined as the amount of radioactivity remaining after incubation of membrane protein in the presence of excess unlabeled compound. Protein concentration may be measured by the Bradford method using Bio-Rad Reagent, with bovine serum albumin as a standard.

Cyclic AMP (cAMP) Formation Assay

[0382] The receptor-mediated inhibition of cyclic AMP (cAMP) formation may be assayed in transfected cells expressing the mammalian receptors described herein. Cells are plated in 96-well plates and incubated in Dulbecco's phosphate buffered saline (PBS) supplemented with 10 mM HEPES, 5 mM theophylline, 2 μg/ml aprotinin, 0.5 mg/ml leupeptin, and 10 μg/ml phosphoramidon for 20 min at 37° C., in 5% CO_2. Test compounds are added and incubated for an additional 10 min at 37° C. The medium is then aspirated and the reaction stopped by the addition of 100 mM HCl. The plates are stored at 4° C. for 15 min, and the cAMP content in the stopping solution measured by radioimmunoassay. Radioactivity may be quantified using a gamma counter equipped with data reduction software.

Generation of Chimeric G-proteins

[0383] Chimeric G-proteins were constructed using standard mutagenesis methods (Conklin et al., 1993). Two chimeras were constructed. The first comprises the entire coding region of human $G\alpha_q$ with the exception of the final 3' 15 nucleotides which encode the C-terminal 5 amino acids of $G\alpha_{i3}$. The second also comprises the entire coding region of human $G\alpha_q$ with the exception of the final 3' 15 nucleotides which encode the C-terminal 5 amino acids of $G\alpha_z$. Sequences of both chimeric G-protein genes were verified by nucleotide sequencing. For the purposes of expression in oocytes, synthetic mRNA transcripts of each gene were synthesized using the T7 polymerase.

Phosphoinositide Assay

[0384] The agonist activities of GABA-B agonists were assayed by measuring their ability to generate phosphoinositide production in COS-7 cells transfected transiently with $GABA_BR1$, $GABA_BR2$, and chimeric $G\alpha_{q/z}$. Alternatively, COS-7 cells are transfected transiently with $GABA_BR$ 1, $GABA_BR2$, and other chimeric G-protein alpha subunits such as $G\alpha_{q/i2}$, $G\alpha_{q/i3}$, or $G\alpha_{q/o}$. Cells were plated in 96-well plates and grown to confluence. The day before the assay the growth medium was changed to 100 μl of medium containing 1% serum and 0.5 μCi [^{3}H]myo-inositol, and the plates were incubated overnight in a CO_2 incubator (5% CO_2 at 37° C.).

[0385] Immediately before the assay, the medium was removed and replaced by 200 μl of PBS containing 10 mM LiCl, and the cells were equilibrated with the new medium for 20 min. The [^{3}H]inositol-phosphate (IP) accumulation was started by adding 22 μl of a solution containing the agonist. To the first two wells 22 μl of PBS were added to measure basal accumulation, and 10 different concentrations of agonist were assayed in the following 10 wells of each plate row. All assays were performed in duplicate by repeating the same additions in two consecutive rows. The plates were incubated in a CO_2 incubator for 30 min. The reaction was terminated by removal of the buffer solution by blotting, followed by the addition of 100 μl of 50% (v/v) trichloroacetic acid (TCA), and 10 min incubation at 4° C.

[0386] The contents of the wells were then transferred to a Multiscreen HV filter plate (Millipore) containing Dowex AG1-X8 (200-400 mesh, formate form). The filter plates were prepared adding 100 μl of Dowex AG1-X8 suspension (50% v/v, water:resin) to each well. The filter plates were placed on a vacuum manifold to wash or elute the resin bed. Each well was washed 3 times with 200 μl of 5 mM myo-inositol. The [^{3}H]-IPs were eluted into empty 96-well plates with 75 μl of 1.2 M ammonium formate/0.1 M formic acid. After the addition of 200 μl of scintillation cocktail (Optiphase Supermix; Wallac) to each well, [^{3}H]-Ips were quantified by counting on a Trilux 1450 Microbeta scintillation counter.

Oocyte Expression

[0387] Female *Xenopus laevis* (Xenopus-1, Ann Arbor, Mich.) are anesthetized in 0.2% tricain (3-aminobenzoic acid ethyl ester, Sigma Chemical Corp.) and a portion of ovary is removed using aseptic technique (Quick and Lester, 1994). Oocytes are defolliculated using 3 mg/ml collagenase (Worthington Biochemical Corp., Freehold, N.J.) in a solution containing 87.5 mM NaCl, 2 mM KCl, 2 mM $MgCl_2$ and 5 mM HEPES, pH 7.5. Oocytes are injected (Nanoject, Drummond Scientific, Broomall, Pa.) with 50-70 nl mRNA prepared as described below. After injection of mRNA, oocytes are incubated at 17 degrees for 3-8 days.

[0388] RNAs are prepared by transcription from: (1), linearized DNA plasmids containing the complete coding region of the gene, or (2), templates generated by PCR incorporating a T7 promoter and a poly A^+ tail. From either source, DNA is transcribed into mRNA using the T7 polymerase ("Message Machine", Ambion).

[0389] The transcription template for the rat $GABA_BR1b$ gene was prepared by PCR amplification of the plasmid BO58 using the primers MJ23 and MJ47 (see below). The template for the rat $GABA_BR2$ gene was made by linearization of the plasmid BO56, rat $GABA_BR2$ insert from BO55 in the expression vector pEXJ.T7, with NotI.

[0390] Primers:

MJ23 5' CCAAGCTTCTAATACGACTCACTATAGGGGAGACCATGGGCCCGGGGGG (SEQ ID NO:30); ACCCTGTACC 3'

MJ47 5' $T_{(35)}$CACTTGTAAAGCAAATGTACTCGACTCC 3' (SEQ ID NO:31).

[0391] Genes encoding G-protein inwardly rectifying K^+ channels 1 and 4 (GIRK1 and GIRK4; "GIRKs") were obtained by PCR using the published sequences (Kubo et al., 1993; Dascal et al., 1993; Krapivinsky et al., 1995b) to derive appropriate 5' and 3' primers. Human heart cDNA was used as template together with the primers

5' -CGCGGATCCATTATGTCTGCACTCCGAAGGAAATTTG-3' (SEQ ID NO:32) and

5' -CGCGAATTCTTATGTGAAGCGATCAGAGTTCATTTTTC-3' (SEQ ID NO:33) for GIRK1 and

5' -GCGGGATCCGCTATGGCTGGTGATTCTAGGAATG-3' (SEQ ID NO:34) and

5' -CCGGAATTCCCCTCACACCGAGCCCCTGG-3' (SEQ ID NO:35) for GIRK4.

[0392] The BamH1 and EcoR1 restriction sites in each primer pair were used to clone the PCR product into the expression vector pcDNA-Amp (Invitrogen). Plasmid vectors containing GIRK1 and GIRK4 are referred to as "JS1800" and "JS1741", respectively. The coding regions of both genes were sequenced and verified.

Oocyte Electrophysiology

[0393] Dual electrode voltage clamp ("GeneClamp", Axon Instruments Inc., Foster City, Calif.) is performed using 3 M KCl-filled glass microelectrodes having resistances of 1-3 Mohms. Unless otherwise specified, oocytes are voltage clamped at a holding potential of −80 mV. During recordings, oocytes are bathed in continuously flowing (1-3 ml/min) medium containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.5 (ND96), or elevated K^+ containing 49 mM KCl, 49 mM NaCl, 1.8 mM $CaCl_2$, 2 mM $MgCl_2$, and 5 mM HEPES, pH 7.5 (hK). Drugs are applied either by local perfusion from a 10 μl glass capillary tube fixed at a distance of 0.5 mm from the oocyte, or for calculation of steady-state EC_{50}s, by switching from a series of gravity fed perfusion lines. Experiments are carried out at room temperature. All values are expressed as mean +/− standard error of the mean.

[0394] Concentration-response curves for agonists and antagonists were fitted with logistic equations of the form $I=1/(1+(EC_{50}/[Agonist])^n)$ for agonists and $I=1/(1+([Antagonist]/IC_{50})^n)$ for antagonists, where I is current, where EC_{50} is the concentration of agonist that produced half-maximal activation, IC_{50} is the concentration of antagonist that produced half-maximal inhibition, and n the Hill coefficient. Fits were made with a Marquardt-Levenberg non-linear least-squares curve fitting algorithm.

Recording Ion Currents in Mammalian Cells

[0395] The ability of the rat $GABA_BR1$ and $GABA_BR2$ genes to activate GIRK currents in mammalian cells was investigated by transient transfection of HEK-293 cells followed by voltage clamp analysis of currents. HEK-293 cells were maintained in Dulbecco's modified Eagle medium (DMEM) plus 10% (v/v) bovine calf serum, 2% L-glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin and were incubated at 37° C. in a humidified 5% CO_2 atmosphere. Cells were harvested twice each week by treatment with 0.25% trypsin/1 mM EDTA in Hank's Salts and re-seeded at 20% of their original density either into 75 cm^2 flasks (for passaging) or into 35 mm tissue culture dishes (for transfection and electrophysiology experiments).

[0396] HEK-293 cells, 40%-80% confluent, were co-transfected with various combinations of 0.6 ug each of the following plasmids: pGreen Lantern-1 (Gibco/BRL, Gaithersburg, Md.), human GIRK1 (JS1800), human GIRK4 (JS1741), rat $GABA_BR1b$ (BO58), and rat $GABA_BR2$ (BO55). Cells were transiently transfected using the Superfect Transfection Reagent from Qiagen (Valencia, Calif.) according to the manufacturer's instructions. Briefly, 3 μg total plasmid DNA were incubated with 22.5 μl Superfect Reagent in 100 μl serum-free DMEM for 5-10 minutes at room temperature. After addition of 600 μl complete DMEM, the DNA/Superfect mixture was transferred to cells growing in 35 mm dishes coated with poly-D-lysine and incubated for 2-4 hours at 37° C. in a 5% CO_2 incubator. Subsequently, the dishes were washed once with phosphate-buffered saline and 2 ml complete DMEM was added. Cells were incubated for 24-72 hours at 37° C. before performing electrophysiological measurements.

[0397] The whole-cell configuration of the patch-clamp technique was used with glass pipettes having resistances of 2-4 MΩ when filled with the pipette solution. Solutions used were (in mM), $KMeSO_4$, 125; KCl, 5; NaCl, 5; $MgCl_2$, 2; EGTA, 11; HEPES, 10, pH 7.4; MgATP, 1.0; Na_2GTP, 0.2, for the pipette and NaCl, 130; KCl, 4; $CaCl_2$, 2; $MgCl_2$, 2; Glucose, 10; Sucrose, 10; HEPES, 10, pH 7.4 for the bath. GIRK currents were recorded in elevated K^+ solution containing 25 mM K^+ and a correspondingly lower concentration of Na^+. Voltage clamp recordings were made with an EPC-9 amplifier using Pulse+PulseFit software (HEKA Elektronik). Series resistances were kept below 10 Mohm and no attempt was made to provide series resistance compensation. Currents were low-pass filtered at 1 kHz and digitized at a rate of 5 kHz. Unless otherwise noted, experiments were performed at room temperature on cells voltage clamped at a holding potential of −70 mV. Application of agonists was realized using a gravity-fed, perfusion system consisting of six concentrically arranged microcapillary tubes (Jones et al. 1997). The time to complete solution exchange was about 100 ms. The bath was constantly perfused at a low rate with control solution.

[0398] All voltage clamp recordings were made from transfected cells visualized under epifluorescent lighting conditions utilizing a filter set designed for GFP (Zeiss Optics) Fluorescent cells were an excellent indication of transfection since they all exhibited some constitutive GIRK current activity in contrast to untransfected cells which displayed no measurable inward rectifier K^+ currents (data not shown).

Microphysiometry

[0399] $GABA_BR1$, $GABA_BR2$ or the combination, were transiently expressed in CHO-K1 cells by liposome mediated transfection according to the manufacturer's recommendations ("LipofectAMINE", GibcoBRL, Bethesda, Md.), and maintained in Ham's F-12 medium with 10% bovine serum. Cells were prepared for microphysiometric recording as previously described (Salon, J. A., et al., 1995). On the day of the experiment the cell capsules were trans-

ferred to the microphysiometer and allowed to equilibrate in recording media (low buffer RPMI 1640, no bicarbonate, no serum, Molecular Devices Corp.), during which a baseline was established. The recording paradigm consisted of a 100 ml/min flow rate and a 30 s flow interruption during which the rate measurement was taken. Challenges involved an 80 s drug exposure just prior to the first post-challenge rate measurement being taken, followed by two additional pump cycles. Acidification rates reported are expressed as a percentage increase of the peak response over the baseline rate observed just prior to challenge.

N-terminal Deletion Experiments

[0400] As a start to exploring the structural aspects of $GABA_BR2$ important for functional activity of the $GABA_BR1/R2$ receptor, N-terminal deletion experiments were performed on the $GABA_BR2$-HA construct (see below). All such deletion mutants caused a complete disruption of receptor activity as assessed by the measurement of GIRK currents in transfected HEK293 cells. In one such experiment, wildtype $GABA_BR2$-HA was digested with BglII restriction enzyme and religated. The BglII deletion mutant (M118) lacks 257 amino acids at the N-terminus, corresponding to positions 226-482. Using immunofluorescence, M118 was found to be expressed on the cell surface, similarly to the wildtype $GABA_BR2$-HA, yet when co-expressed with $GABA_BR1$ did not produce GIRK activation with 100 μM GABA. Thus, although we cannot yet identify specific amino acids contributing to receptor activity, it appears that the N-terminal region comprising amino acids 226-482 is critically important either for dimer formation, ligand binding or conformational changes associated with signal transduction.

Construction of Epitope-tagged Polypeptides and Confocal Microscopy

[0401] Incorporation of sequences encoding the RGS6xHis or influenza virus hemagglutinin (HA) epitope into the $GABA_BR1$ and $GABA_BR2$ genes, respectively, was performed by PCR. Each epitope was positioned immediately before the stop codon in the appropriate gene. Both tagged genes were subcloned into pcDNA. Sequence analysis was used to confirm all PCR-derived portions of the construct. Forty-eight hours post-transfection HEK293 cells were fixed for 20 min in 4% paraformaldehyde in PBS, permeablized in PBS containing 2% BSA and 0.1% Triton X-100 and incubated with primary antibody for 1.5 h. Mouse monoclonal anti-RGS (Qiagen) and mouse anti-FLAG (Boehringer-Mannheim) were labeled with FITC-conjugated goat anti-mouse antibodies. Rat monoclonal anti-HA (Boehringer-Mannheim) was visualized with TRITC-conjugated rabbit anti-irat antibodies. Fluorescent images were obtained with a Zeiss LSM 410 confocal microscope using a 100× oil-immersion objective.

Immunoprecipitation and Western Blotting

[0402] Forty-eight hours following transient transfection HEK293 cells were solubilized in lysis buffer containing (in mM): 50 Tris/Cl pH 7.4, 300 NaCl, 1.5 $MgCl_2$, 1 $CaCl_2$, protease inhibitors (Boehringer Mannheim tablets), 1% Triton X-100, and 10% glycerol. 1-2 mg of protein was immunoprecipitated overnight at 4° C. with either 0.5 μg rat monoclonal anti-HA antibody or 0.5 μg mouse monoclonal anti-4xHis antibody (Qiagen). Immune complexes were bound to 20 μl Protein-A agarose (Research Diagnostics, Inc.) for 2 h at RT. Protein-A pellets were washed twice with buffer containing Triton-X-100, then once without, and eluted with 80 μl Laemmli sample buffer containing 2% (w/v) SDS and 20 mM DTT. After heating for 3 min. at 70° C., 20 μl IP samples or 20 μg total protein was subjected to SDS-PAGE followed by Western blotting with either anti-HA or anti-4xHis antibody, followed by sheep anti-rat (Amersham) or goat anti-mouse (RDI) HRP-linked secondary antibodies, respectively. Proteins were visualized with enhanced chemiluminescent substrates (Pierce).

[0403] Alternatively, material for immunoprecipitations was obtained by sucrose gradient fractionation of the P1 pellet as described by Graham(Graham, 1984). To verify the enrichment of plasma membrane in the resulting "P1+" pellet, Na^+/K^+ ATPase in the P1+ and P2 (primarily microsomal and vesicular(Graham, 1984)) fractions was quantified by fluorescence detection of anti-alpha 1 subunit antibody (Research Diagnostics, Inc., clone 9A-5) on a phosphor imager (Molecular Dynamics). ATPase in P1+ fractions used for immunoprecipitations was found to be enriched >50 fold compared to P2 fractions.

Experimental Results

Novel GPCR Sequences Identified by BLAST Search

[0404] The rat $GABA_BR1a$ amino acid sequence (Kaupmann et al. (1997) Nature 386:239) was used as a query to search the EST division of GenBank with BLAST. Two entries, T07621 and Z43654, had probability scores that suggested significant amino acid homology to the $GABA_BR1a$ polypeptide. T07621 had sequence homology from the beginning of the first transmembrane domain to the beginning of third transmembrane domain of the $GABA_BR1a$ polypeptide. Z43654 had sequence homology from the sixth transmembrane domain to the seventh transmembrane domain of the $GABA_BR1a$ polypeptide. The sequence documentation for T07621 and Z43654 was retrieved with Entrez (NCBI) and neither sequence was annotated as having homology to any 7-TM spanning protein.

[0405] These results were used to obtain a full-length human clone TL-266, comprising both of the sequences identified by the BLAST search. Sequence analysis of clone TL-266 revealed a complete coding region for a novel protein. A search of the GenEMBL database indicated that the most similar sequence was that of $GABA_BR1a$, followed by G protein-coupled receptors (GPCRs) of the metabotropic receptor superfamily. The nucleotide and deduced amino acid sequence of TL-267 are shown in **FIGS. 1 and 2**, respectively. The nucleotide sequence of the coding region is 57% identical to the rat $GABA_BR1a$ over a region of 1,686 bases. The longest open reading frame encodes an 898 amino acid protein with 38% amino acid identity to the rat $GABA_BR1a$ polypeptide. Hydropathy plots of the predicted amino acid sequence reveal seven hydrophobic regions that may represent transmembrane domains (TMs, data not shown), typical of the G protein-coupled receptor superfamily. In the putative TM domains, $GABA_BR2$ exhibits 45% amino acid identity with the rat $GABA_BR1a$ polypeptide. The amino terminus of TL-266

has amino acid homology to the bacterial periplasmic binding protein, a common feature of the metabotropic receptors (O'Hara et al. (1993) Neuron 11:41-52).

Generation of rat $GABA_BR2$ PCR Product

[0406] Using PCR primers designed against the first and seventh transmembrane domains of the human $GABA_BR2$ sequence, BB257 and BB258, a 780 base pair fragment was amplified from rat hippocampus and rat cerebellum. Sequence from these bands displayed 90% nucleotide identity to the human $GABA_BR2$ gene. This level of homology is typical of a species homologue relationship in the GPCR superfamily.

Construction and Screening of a Rat Hypothalamic cDNA Library

[0407] To obtain a full-length rat $GABA_BR2$ clone, pools of a rat hypothalamic cDNA library were screened by PCR using primers BB265 and BB266. A 440 base pair fragment was amplified from 44 out of 47 pools. Vector-anchored PCR was performed to identify pools with the largest insert size. One positive primary pool, 1-47, was subdivided into 24 pools of 1000 individual clones and screened by vector-anchored PCR. Seven positive subpools were identified and one, 1-47-4, was subdivided into 10 pools of 200 clones, plated onto agar plates, and screened by southern analysis. Four closely clustering colonies that appeared positive were rescreened individually by vector-anchored PCR. One positive colony, 1-47-4-2, designated BO54, was amplified as a single rat $GABA_BR2$ clone. Since vector-anchored PCR revealed that BO54 was in the wrong orientation for expression, the insert was isolated by restriction digest and subcloned into the expression vector pEXJ. A transformant in the correct orientation was identified by vector-anchored PCR, and designated BO-55.

[0408] The nucleotide and deduced amino acid sequence of BO-55 are shown in **FIGS. 3 and 4**, respectively. BO-55 contains a 2.82 kB open reading frame and encodes a polypeptide of 940 amino acids. The nucleotide sequence of BO-55 is 89% identical to TL-267 in the coding region, with an overall amino acid identity of 98%. The proposed signal peptide cleavage site is between amino acids 40 and 41 (Nielsen et al., 1997).

[0409] A BLAST search of GenEMBL indicated that this sequence was most closely related to $GABA_BR1$, displaying 35% and 41% amino acid identities overall and within the predicted transmembrane domains, respectively (**FIG. 10**). The structural similarity to $GABA_BR1$ indicated that this sequence encodes a novel polypeptide, which we refer to as $GABA_BR2$. The next most related sequences were other members of the mGluR family, with 21-24% overall amino acid identities. Like $GABA_BR1$ and other members of the mGluR family (O'Hara, P. J., et al., 1998), $GABA_BR2$ contains a large N-terminal extracellular domain having regions of homology to bacterial periplasmic binding proteins.

Distribution of $GABA_BR1$ or $GABA_BR2$ in cDNA Libraries

[0410] Three cDNA libraries were screened by PCR with primers directed to transmembrane regions of either $GABA_BR$ 1 or $GABA_BR2$. In a human hippocampal cDNA library both polypeptides were found in greater than 90% of the pools and in a rat hypothalamic cDNA library, again both polypeptides were found in greater than 90% of the pools. In addition, within each of these two libraries, the respective frequency of $GABA_BR$ L and $GABA_BR2$ seems to be the same. However, in a rat spinal cord cDNA library, $GABA_BR1$ was found in 62.5% of the pools while $GABA_BR2$ was found in only 17.5% of the pools. Thus, while both polypeptide subtype appear to be present at high frequency in all three of the libraries, in the spinal cord library $GABA_BR2$ occurs at 3.6-fold lower frequency. These data point to the existence of an additional $GABA_B$-like peptide(s).

Results of Localization Controls

[0411] The specificity of the hybridization of the $GABA_BR2$ probe was verified by performing in situ hybridization on transiently transfected HEK293 cells as described in Methods. The results indicate that hybridization to each of the individual $GABA_B$polypeptides was specific only to the HEK293 cells transfected with each respective cDNA. In addition, in situ hybridization on rat brain sections was performed using two hybridization probes targeted to different segments of the $GABA_BR2$ mRNA. In each case the pattern and intensity of labeling was identical in all regions of the rat CNS. Nonspecific hybridization signal was determined using the sense probes and was indistinguishable from background.

Localization of $GABA_BR2$ mRNA in Rat CNS

[0412] The anatomical distribution of $GABA_BR2$ mRNA in the rat brain was determined by in situ hybridization. By light microscopy the silver grains were determined to be distributed over neuronal profiles. The results suggest that the mRNA for $GABA_BR2$ is widely distributed throughout the rat CNS in addition to several sensory ganglia (FIGS. 19H-I) . However, expression levels in the brain were not uniform with several regions exhibiting higher levels of expression such as the medial habenula, CA3 region of the hippocampus, piriform cortex, and cerebellar Purkinje cells (FIGS. 19A-F). Moderate expression levels were observed in the ventral pallidum, septum, thalamus, CA1 region of the hippocampus, and geniculate nuclei (**FIGS. 19C, D, E**). Lower expression of $GABA_BR2$ mRNA was seen in the hypothalamus, mesencephalon, and several brainstem nuclei (**FIGS. 19D, F**). GABAergic neurons and terminals are likewise widely distributed in the CNS (Mugnaini, E., et al., 1985). and the distribution of the $GABA_BR2$ mRNA correlates well with the distribution of GABAergic neurons. One exception is the substantia nigra which contains high densities of GABAergic neurons, yet very low expression of $GABA_BR2$ mRNA. Additionally, the anatomical distribution of $GABA_BR2$ mRNA is in concordance with previous reports of the distribution of $GABA_B$binding sites obtained using [^{3}H]baclofen (Gehlert, D. R., et al., 1985), and [^{3}H] GABA (Bowery, N. J., et al., 1987). Furthermore, there was a high degree of similarity in the distribution and intensity of $GABA_BR2$ hybridization signal relative to those previously reported for $GABA_BR1$ (Bischoff, S., et al., 1997) (**FIGS. 11, 12**). Notable exceptions were the hypothalamus and caudate-putamen, where the expression of $GABA_BR2$ message appeared lower than that of $GABA_BR1$.

Colocalization of $GABA_BR2$ and $GABA_BR1b$ mRNAs in the Rat CNS

[0413] The results of the in situ hybridization studies using digoxygenin-labeled probe conjugated to alkaline phosphatase and the chromagen NBT/BCIP for the $GABA_BR2$ mRNA and an [^{35}S]dATP-labeled probe for the $GABA_BR1b$ mRNA indicated that coexpression of the $GABA_BR2$ mRNA and $GABA_BR1b$ mRNA did occur in vivo in neurons. In particular, colocalization was observed in cells of the medial habenula, hippocampus, and the cerebellar Purkinje cells. Likewise, there was evidence from the autoradiograms for potential overlapping distribution of the three known $GABA_B$mRNAs in the olfactory bulb, throughout the entire neocortex, several hypothalamic nuclei, numerous thalamic nuclei and brain stem nuclei. However, the Purkinje cells of the cerebellum contained message for only $GABA_BR2$ and $GABA_BR1b$ and not the $GABA_BR1a$. Additionally, all three subtypes appear to be distributed throughout the gray matter of the spinal cord in all levels of the spinal cord.

[0414] The overlapping expression patterns of $GABA_BR1$ and $GABA_BR2$ transcripts in the brain suggested the possibility the polypeptides may be co-expressed in individual neurons and that both might be required for functional activity.

Oocyte Expression

[0415] Postsynaptic inhibition of neurons by $GABA_B$ receptor activation is caused by the opening of inwardly rectifying K^+ channels (GIRK) (North, R. A., 1989; Andrade, R. et al., 1986; Gahwiler, B. H., et al., 1985; Luscher, C., et al., 1997). Oocytes expressing the combination of $GABA_BR1b$ and $GABA_BR2$ mRNAs together with GIRKs elicited large currents in response to 30 μM GABA (Table 1a and 1b). (Subsequent to the compilation of the data in Table 1a, experiments were done to make Table 1b.) GABA and baclofen evoked sustained currents of similar magnitude (**FIG. 13B**). In contrast, oocytes expressing transcripts encoding either $GABA_bR1a$, $GABA_BR1b$, or $GABA_BR$ 2 alone consistently failed to generate GIRK currents in response to high concentrations of GABA (1 mM), baclofen (1 mM) or 3-APMPA (100 μM). Others have reported similar results with $GABA_BR1$ (Kaupmann, K. et al., 1997a; Kaupmann, K., et al., 1997b).

TABLE 1a

Magnitude of GIRK currents stimulated by GABA in oocytes and HEK-293 cells expressing GIRK1 and GIRK4 and various combinations of rat $GABA_BR1$ and rat $GABA_BR2$.

	Oocytes			HEK-293		
	mean (nA)	S.E.M.	(n)	mean (pA)	S.E.M.	(n*)
$GABA_BR1a$	0	0	(35)	—	—	—
$GABA_BR1b$	0	0	(15)	5	3	(3/26)
$GABA_BR2$	0	0	(19)	5	5	(1/6)
$GABA_BR1b$+ $GABA_BR2$	1396	269	(7)	658	323	(9/10)
$GABA_BR1b$+ $GABA_BR2$+ PTX	7	7	(2)	—	—	—

*number of cells responding/total number studied

[0416]

TABLE 1b

Magnitude of GIRK currents stimulated by GABA in oocytes and HEK-293 cells expressing GIRK1 and GIRK4 and various combinations of rat $GABA_BR1$ and rat $GABA_BR2$.

	Oocytes			HEK-293		
	mean (nA)	S.E.M.	(n)	mean (pA)	S.E.M.	(n*)
$GABA_BR1a$	0	0	(35)	—	—	—
$GABA_BR1b$	0	0	(23)	5	3	(5/26)
$GABA_BR2$	0.230	.13	(30)	.87	.87	(1/23)
$GABA_BR1b$+ $GABA_BR2$	832	65	(65)	470	71	(70/81)
$GABA_BR1b$+ $GABA_BR2$+ PTX	16	9	(3)	—	—	—

*number of cells responding/total number studied

[0417] Currents stimulated by GABA in oocytes injected with both $GABA_BR1b$ and $GABA_BR2$ mRNAs were completely blocked by the selective antagonist CGP55845 (1 μM) in a reversible fashion (data not shown). The potency of GABA and baclofen for eliciting GIRK currents was measured by performing steady-state cumulative concentration response assays on individual oocytes (**FIG. 6A**). Like K^+ responses elicited by stimulation of native $GABA_B$ receptors (Lacy et al. 1988; Misgeld et al. 1995), responses in oocytes did not desensitize and could be faithfully reproduced by multiple agonist applications on single oocytes. Stimulation of inward current was concentration dependent for both GABA and baclofen. The EC_{50}s, 1.76 μM for GABA and 3.99 μM for baclofen (**FIG. 6B, FIG. 7**), agreed closely with those reported in the literature for native receptors (Lacy et al. 1988; Misgeld et al. 1995). Concentration-effect curves for GABA were shifted to the right, in an apparently competitive manner, by well characterized $GABA_B$-selective antagonists (**FIG. 15B**). Based on additional experiments, the EC_{50}'s are 1.32 μM for GABA and 3.31 μM for baclofen. The results to date are summarized in Table 2. Antagonist affinity estimates (**FIG. 15B**, Table 2) were similar to values reported in previous electrophysiological studies using brain tissue (Bon, C., et al., 1996; Seabrook, G. R., et al., 1990), as well as to those obtained by measuring displacement of radioligand from cells expressing $GABA_BR1$ alone (Kaupmann, K., et al., 1997a) (Table 2).

TABLE 2

Agonist and antagonist pharmacology in cells expressing $GABA_BR1$, $GABA_BR2$, or both.

	Protein Measurement Agonist				Antagonist		
		GABA	Baclofen	3-APMPA	Phaclofen	CGP54626	CGP55845
$GABA_BR1$ + $GABA_BR2$	pEC_{50}[1], pK_e[2]	5.88 ± 0.01	5.48 ± 0.05	7.29 ± 0.02	3.80 ± 0.03[4]	7.48 ± 0.05	8.60 ± 0.09
$GABA_BR1$	pK_i[3]	4.6	4.3	5.2	>3.0	8.95	8.7

[1]n = 6–8 oocytes except for GABA; n = 20 oocytes.
[2]Measured using GABA as agonist; n = 4–6 oocytes.
[3]Displacement of [^{3}H]-CGP54626 from COS-7 cells expressing $GABA_BR1$; n = 3.4
[4]IC_{50} using EC_{50} concentration of GABA.

[0418] Evidence that GABA-induced currents were mediated by GIRK channels included: 1) dependency on elevated external K^+, 2) strong inward rectification of the current-voltage (I/v) relation, 3) reversal potential (−23.3 mV) close to the predicted equilibrium potential for K^+ (−23 mV), and 4) sensitivity to block by 100 μM Ba^{++} (**FIG. 8**).

[0419] Three oocytes were injected with pertussis toxin (2 ng/oocyte) 6 h before voltage clamping. GABA-stimulated currents were abolished in these oocytes (Table 1a and 1b), suggesting that receptor activation of GIRKs was mediated by G-proteins G_i or G_o. Analogous results have been obtained by others expressing D2 dopamine receptors with GIRKs in oocytes (Werner et al. 1996).

GABA Responses in Co-transfected HEK-293 Cells

[0420] To verify that both gene products, $GABA_BR1b$ and $GABA_BR2$, are also required for expression of functional $GABA_B$ receptors in mammalian cells, voltage clamp recordings were obtained from HEK-293 cells transiently transfected with various combinations of each gene along with GIRKs. Cells transfected with a combination of $GABA_BR1b$ (BO58) and $GABA_BR2$ (BO55) plus GIRKs consistently produced large K^+ currents in response to 100 μM GABA (9 of 10 cells tested, Table 1a and 70 of 81 cells tested, Table 1b). Large amplitude currents were also observed when $GABA_BR2$ was paired with the $GABA_BR1a$ splice variant (1046" 247 pA; n=9). In contrast, cells transfected with only one of the $GABA_B$ genes plus GIRKs responded either not at all or only very weakly to GABA (Table 1a and 1b). Small agonist-evoked currents (10-50 pA) were observed in 5 of 26 cells expressing $GABA_BR1$; similar weak currents were evoked in 1 of 23 cells expressing $GABA_BR2$.

[0421] GABA-elicited currents in doubly transfected cells were completely blocked by 100 μM Ba^{++} or the competitive antagonist CGP55845 at 1 μM (**FIG. 9**) . The EC_{50} for GABA stimulation of GIRKs in HEK-293 cells was determined using similar methods as for oocytes. The EC_{50}, 3.42 μM, was comparable to that measured in oocytes (1.76 μM; further experiments gave 1.32 μM). Thus, whether in Xenopus oocytes or HEK-293 cells, the behavior of the $GABA_B$ receptor is the same. Co-expression of both $GABA_BR1b$ and $GABA_BR2$ is required to observe activation of the receptor by GABA.

[0422] To determine if co-expressed $GABA_BR1/R2$ could mediate a cellular response in the absence of exogenously supplied GIRKs, we transiently co-transfected CHO cells with $GABA_BR1$ and $GABA_BR2$ and measured agonist-evoked extracellular acidification using a microphysiometer. Baclofen stimulated a 9-fold increase in acidification rate (**FIG. 16**) which was blocked by 100 nM CGP55845 and by pretreatment with PTX (not shown). This response was absent in cells expressing either protein alone. Since GIRK activity is undetectable in wild-type CHO cells (Krapivinsky, G., et al., 1995b) we conclude that GIRK expression is not a prerequisite for signal generation by $GABA_BR1/R2$.

$GABA_BR1/GABA_BR2$ Signaling Through Chimeric G-proteins

[0423] Chimeric G-proteins have been used to "switch" the coupling pathway of a GPCR from one that normally inhibits adenylyl cyclase to one that activates phospholipase C (Conklin et al., 1993). With the aim of developing an assay based on Ca^{++} or some other signal amenable to high throughput screening, we employed a $G\alpha_{q/i3}$ chimera to obtain Ca^{++}-induced Cl^- responses in oocytes. Oocytes were injected with $GABA_BR1$ and $GABA_BR2$ mRNAs as previously described. 2-3 days later oocytes were injected again with 50 pg of $G\alpha_{q/i3}$ mRNA and recorded under voltage clamp conditions. In response to GABA (0.1-1 mM) 88% of these oocytes produced rapidly desensitizing inward currents (454±92 nA; n=14) typical of those stimulated by receptors that normally couple to $G\alpha_q$. In contrast, oocytes injected with only the $GABA_BR1/GABA_BR2$ combination (n>100), or $GABA_BR1$ plus $G\alpha_{q/i3}$ (n=4) failed to produce currents.

[0424] $GABA_B$ agonists also resulted in concentration-dependent stimulation of phosphoinositide production in COS-7 cells transfected transiently with $GABA_BR1$, $GABA_BR2$, and the chimeric G-protein $G\alpha_{q/z}$. The concentration of agonist evoking 50% of its maximum response (EC_{50}) and fold stimulation over basal were: GABA (EC_{50}= 1.8 μM; 2.4 fold); baclofen (1.7 μM; 1.8 fold); 3-aminopropylmethylphosphinic acid (EC_{50}=0.11 μM; 2.2 fold). These results indicate that G-protein chimeras, in particular $G\alpha_{q/z}$ and $G\alpha_{q/i3}$, are useful for directing $GABA_B$ receptor stimulation to a phosphoinositide- or Ca^{++}-based assay.

[0425] A comparison of the pharmacological properties of $GABA_BR1$ and $GABA_BR2$ using radioligand binding revealed that membranes from HEK293 or COS-7 cells expressing $GABA_BR1$, but not those expressing $GABA_BR2$, were labeled by the high affinity antagonist [^{3}H]-CGP54626[21] (Table 2), indicating that the polypeptides are pharmacologically distinct. Neither was labeled by the agonists [^{3}H]-GABA or [^{3}H]-baclofen. Furthermore, with the available ligands (GABA, baclofen, APMPA, phaclofen, CGP54626, CGP-55845 and SCH-50911) the binding profile of membranes from cells co-transfected with $GABA_BR1/R2$ was not different from those transfected with $GABA_BR1$ alone. The absence of detectable high affinity agonist binding to $GABA_BR1/R2$, as well as to $GABA_BR1b$, constitutes a notable distinction from the $GABA_B$ binding profile in the CNS and may reflect the absence of an essential, as yet undefined G-protein or accessory protein.

[0426] The molecular mechanism by which protein co-expression confers functional activity is unknown. We noted that varying the ratios of $GABA_BR1/R2$ cDNAs from 1/100 to 100/1 in HEK293 cells resulted in a symmetrical fall off in response amplitude (**FIG. 14B**). This suggests that a 1:1 protein stoichiometry may be critical, and caused us to postulate that the polypeptides are forming a heteromeric association. Biochemical evidence supports the idea that certain GPCRs can exist as homodimers (Hebert, T. E., et al.,

1996; Cvejic, S., et al., 1997; Ciruela, F., et al., 1995; Avissar, S., et al., 1983; Romano, C., et al., 1996), but the functional significance of this has been largely unexplored (Hebert, T. E., et al., 1996; Wreggett, K. A., et al., 1995). The possibility of a physical association was investigated using epitope-tagged versions of $GABA_BR1$ (RGS6xH tag) and $GABA_BR2$ (HA tag). C-terminal modification did not appear to alter the function of either polypeptide; maximal current amplitudes (**FIG. 14B**) and EC_{50} values for GABA (4.97 μM, n=5) were unchanged compared to HEK293 cells expressing the wild-type $GABA_BR1/R2$ receptor combination (3.42 μM, n=5). The subcellular distribution of epitope-tagged proteins was examined in transfected cells by fluorescence microscopy. When expressed individually, $GABA^BR1^{RGS6xH}$ and $GABA_BR2^{HA}$ were localized throughout the plasma membrane. Optical sectioning of antibody-labeled cells by confocal microscopy confirmed the membrane localization pattern, with less labeling in the cytoplasm and none in the nucleus. In co-transfected cells there was a striking overlap in the distribution of the two epitope tags (**FIG. 17A-17C**). Both proteins were prominently expressed on the plasma membrane. Furthermore, co-localization occurred within the cytoplasm, suggesting that $GABA_BR1$ and $GABA_BR2$ assemble in the endoplasmic reticulum. In contrast, the cellular distribution of an unrelated GPCR, NPY Y5, differed considerably from that of $GABA_BR2$ (**FIG. 17D**), suggesting specificity in the association of $GABA_BR2$ with $GABA_BR1$.

[0427] Western blots of whole cell extracts from cells expressing $GABA_BR1^{RGS6xH}$, $GABA_BR2^{HA}$ or both, exhibited bands close to the predicted molecular weights of the two proteins (92 kD for $GABA_BR1$, 97 kD for $GABA_BR2$) and additional bands corresponding to the predicted molecular weights of receptor dimers (**FIG. 18A,B**). To determine if $GABA_BR1$ and $GABA_BR$ 2 co-associate in a heteromeric complex, we immunoprecipitated solubilized material from cells expressing both polypeptides. $GABA_BR2^{HA}$ was detected in material immunoprecipitated using either anti-His or anti-HA antibodies (**FIG. 18**). To determine if $GABA_BR1b$ and $GABA_BR2$ co-associate in a heteromeric complex, we performed immunoprecipitations using membrane fractions enriched in plasma membrane as determined by the presence of Na^+/K^+ ATPase (**FIG. 20A**). In co-transfected cells only, $GABA_BR2^{HA}$ was detected in material immunoprecipitated using antibodies specific for the $GABA^BR1^{RGS6xH}$ protein (**FIG. 20B**). This result confirms that both $GABA_BR1$ and $GABA_BR2$ are correctly targeted to the plasma membrane of HEK293 cells, and that the two proteins exist in a heteromeric complex, perhaps as heterodimers, on the membrane surface.

Responses Measured Using a Microphysiometer

[0428] The results described so far using rat clones of $GABA_BR1$ and $GABA_BR2$ indicate that heterodimer formation is critical for the activation of $GABA_B$ receptors by agonists. Co-expression experiments with human polypeptides suggest that the same phenomenon occurs for human homologues of $GABA_BR1$ and $GABA_BR2$. For example, using the microphysiometer we found that only when both rat $GABA_BR1$ and rat $GABA_BR2$ were co-expressed was there a dose-dependent acidification response to baclofen. In other experiments when human $GABA_BR1$ was substituted for rat $GABA_BR1$, and co-expressed with rat $GABA_BR2$, a similar concentration-dependent acidification response was observed (**FIG. 24**). The calculated EC_{50} values for baclofen were indistinguishable between rat $GABA_BR1$/rat $GABA_BR2$ (1.09 μM) and human $GABA_BR1$/rat $GABA_BR2$ (1.08 μM). These data strongly support the notion that like the rat receptor, the human $GABA_B$ receptor is formed by subunits composed of $GABA_BR1$ and $GABA_BR2$.

$GABA_B$ Mediated Calcium Responses Measured Using FLIPR

[0429] Confluent monolayers of COS-7 cells in 150 cm^2 flasks were transfected simultaneously with three individual plasmids containing (1) the human $GABA_BR1$ (5 ng), (2) the human $GABA_BR2$ (5 ng), and (3) the chimeric G-protein $G\alpha_{q/13}$ (10 ng), using the DEAE/Dextran method (Lopata, et al., 1984). The cells were grown under controlled conditions for 24 h, at which time they were re-seeded into poly-D-lysine coated 96 well plates (Biocoat, VWR #62406-036) at a density of 50000 cells/well, and grown for another 24 h.

[0430] On the day of the assay, the medium was aspirated and 100 μl of loading buffer (Hank's buffer containing 20 mM HEPES, 1% FBS, 2.5 mM probenecid, and 4 μM Fluo-3 μM; F1241 Molecular Probes, Eugene, Oreg.) was added to each well. The cells were then incubated for 1 h at 37° C. in an atmosphere of 5% CO_2 The cells were then washed 3× with assay buffer (Hank's buffer containing 20 mM HEPES and 2.5 mM probenecid) at room temperature. The cells were left in a final volume of 150 assay buffer. To begin the assay, test compounds were added from drug plates in 50 μl volumes via the FLIPR instrument (Molecular Devices, Sunnyvale, Calif.) in a volume of 50 μl. Receptor-mediated increases in intracellular Ca^+ concentration were recorded as flourescent units. After subtracting baseline fluorescence, responses above background were plotted versus agonist concentration to give final concentration effect curves. Concentration-effect curves were analyzed by non-linear regression analysis to derive agonist potencies.

[0431] The $GABA_B$ agonist 3-APMPA evoked concentration dependent increases in intracellular Ca^+ concentration in COS-7 cells transfected with $GABA_BR1$, $GABA_BR2$, and $G\alpha_{q13}$. The potency derived for 3-APMPA in this assay was 6.85 (pEC_{50}).

Experimental Discussion

[0432] A gene has been cloned that shows 38% overall identity at the amino acid level with the recently cloned $GABA_BR1$ polypeptide. Important predicted features of the new gene product include 7 transmembrane spanning regions, and a large extracellular N-terminal domain. Like the $GABA_BR1$ gene product, $GABA_BR2$ by itself does not promote the activation of cellular effectors such as GIRKs. When co-expressed together, however, the two permit a $GABA_B$ receptor phenotype that is quite similar to that found in the brain. The functional attributes of this reconstituted receptor include: 1) robust stimulation of a physiological effector (GIRKs), 2) EC_{50}s for GABA and baclofen in the same range as for $GABA_B$ receptors previously studied in the CNS, 3) antagonism by the high affinity selective antagonist CGP55845, and 4) inhibition of receptor function by pertussis toxin. These attributes are not observed when either $GABA_BR1$ or $GABA_BR2$ is expressed alone.

[0433] Our data indicate that $GABA_BR1$ and $GABA_BR2$ associate as subunits to produce a single pharmacologically

and functionally defined receptor. Consistent with this view, double labeling in situ hybridization experiments provided evidence that $GABA_BR1$ and $GABA_BR2$ mRNAs are co-expressed in individual neurons and populations of neurons in several regions of the nervous system including hippocampal pyramidal cells (**FIG. 21**), cerebellar Purkinje cells (**FIG. 12A,B**) and sensory neurons in mesencephalic trigeminal nucleus (**FIG. 21**) and dorsal root ganglia. This co-localization pattern of $GABA_BR1$ and R2 transcripts predicts that $GABA_B$ receptors on these cells are comprised of $GABA_BR1/R2$ heteromers. Other as yet unidentified $GABA_B$ receptor homologues may associate elsewhere to produce novel subtypes. For example, the low level of expression of $GABA_BR2$ mRNA relative to $GABA_BR1$ in caudate putamen and hypothalamus (**FIGS. 11A,B**) raises the possibility that other $GABA_B$ receptor homologues may associate with $GABA_BR1$ to produce novel subtypes in these regions. Conclusive evidence that functional $GABA_B$ receptors exist in vivo as multimers will await immunofluorescence studies with specific antibodies.

[0434] The recent cloning of a family of accessory proteins that modify the binding and functional properties of a calcitonin-receptor-like receptor (McLarchie, et al., 1998) demonstrates that some 7-TM spanning proteins require additional unrelated proteins to reconstitute native GPCR activity. $GABA_BR1$ and $GABA_BR2$ are the first examples of 7-TM proteins for which activity is dependent on an interaction with another member within the same family of proteins. There will be considerable interest in whether other GPCRs are formed by heteromeric complexes of related 7-TM proteins. Many members of the superfamily of GPCRs, such as D_3, $5\text{-}HT_5$, and olfactory receptors, do not function well in heterologous expression systems and may require related partners to generate native receptor function (Nimschinsky, et al., 1997). The growing list of receptors that have been reported to exist as homodimers (Ciruela, F., et al., 1995; Cvejic, S., et al., 1997; Hebert, T. E., et al, 1996; Romano, C., et al., 1996; Maggio, R., et al., 1996) points to the likelihood that both homomeric and heteromeric assemblies are more widespread among GPCRs than previously thought.

[0435] There are several possible explanations for why two genes are required for full function of the $GABA_B$ receptor. One possible explanation is that the two gene products function together as a heterodimer having high affinity agonist and antagonist binding sites. Currently, there is no precedent for heterodimerization of GPCRs. There is evidence that certain GPCRs, for example the mGluR5 receptor, can form homodimers via cystine disulfide bridges in the N-terminal domain (Romano et al., 1996).

[0436] Significantly, synthetic peptides that inhibit homodimerization of beta2-adrenergic receptors also reduce agonist stimulation of adenylyl cyclase activity (Hebert et al., 1996). Useful parallels may be drawn from other classes of receptors where heterodimeric structures are well-known. For example, the NMDA (glutamate) receptor is comprised of two principal subunits, neither of which alone permits all of the native features of the receptor (see Wisden and Seeburg, 1993). $GABA_B$ receptors may be comprised similarly of two (or more) peptide subunits, such as $GABA_BR1$ and $GABA_BR2$, that form a quaternary structure having appropriate binding sites for agonist and G-protein.

[0437] A role for $GABA_BR2$ in modulating sensory information is suggested by in situ hybridization histochemistry which revealed the expression of $GABA_BR2$ mRNA in relay nuclei of several sensory pathways. In the olfactory and visual pathways $GABA_BR2$ appears to be in a position to modulate excitatory glutamatergic projections from the olfactory bulb and retina $GABA_BR2$ mRNA was observed in the target regions of projection fibers from the main olfactory bulb, including the olfactory tubercle, piriform and entorhinal cortices and from the retina, for instance the superior colliculus (**FIGS. 19A,B**; Table 3).

[0438] The ability to modulate nociceptive information might be indicated not only by the presence of $GABA_BR2$ transcripts in somatic sensory neurons of the trigeminal and dorsal root ganglia (FIGS. 19H-I) but also by being present in the target regions of nociceptive primary afferent fibers, including the superficial layers of the spinal trigeminal nucleus and dorsal horn of the spinal cord (FIGS. 19F-G). Again, in each of these loci $GABA_BR2$ has been shown to be in a position to potentially modulate the influence of excitatory glutamatergic nociceptive primary afferents. In both ganglia, microscopic examination indicated that the hybridization signal did not appear to be restricted to any one size cell and was distributed evenly over small, medium and large ganglion cells. Thus, $GABA_BR2$ may be able to influence various sensory modalities. Expression levels appeared to be higher in the ganglion cells of the dorsal root with light to moderate expression in the trigeminal ganglia.

[0439] $GABA_BR2$ mRNA was likewise observed to be expressed in the vestibular nuclei which are target regions of inhibitory GABAergic Purkinje cells and also in the Purkinje cells themselves, suggesting that $GABA_BR2$ may be important in the mediation of planned movements (**FIG. 19F**).

[0440] Moderate expression of $GABA_BR2$ transcripts throughout the telencephalon indicate a potential modulatory role in the processing of somatosensory and limbic system (entorhinal cortex) information, in addition to modulating visual (parietal cortex) and auditory stimuli (temporal cortex) as well as cognition. Furthermore, modulation of patterns of integrated behaviors, such as defense, ingestion, aggression, reproduction and learning could also be attributed to this receptor owing to its expression in the amygdala (Table 3). The high levels of expression in the thalamus suggest a possible regulatory role in the transmission of somatosensory (nociceptive) information to the cortex and the exchange of information between the forebrain and midbrain limbic system (habenula). The presence of $GABA_BR2$ mRNA in the hypothalamus indicates a likely modulatory role in food intake, reproduction, the expression of emotion and possibly neuroendocrine regulation (**FIG. 19D**). A role in the mediation of memory acquisition and learning may be suggested by the presence of the $GABA_BR2$ transcript throughout all regions of the hippocampus and the entorhinal cortex (**FIG. 19D**).

TABLE 3

Distribution of rGABA$_B$R2, rGABA$_B$R1a, and GABA$_B$1b mRNA in the rat CNS. The strength of the hybridization signal for each of the respective mRNAs obtained in various regions of the rat brain was graded as weak (+), moderate (++), heavy (+++) or intense (++++) and is relative to the individual polypeptides.

Region	GABA$_B$R2	GABA$_B$R1a*	GABA$_B$R1b*	Potential Application
Olfactory bulb				Modulation of olfactory sensation
internal granule layer	+	++	++	
glomerular layer	+	++	++	
external plexiform layer	—	—	—	
mitral cell layer	—	+	++	
anterior olfactory n	++	++	++	
olfactory tubercle	+	++	+++	
Islands of Calleja	—	++	+++	
Telencephalon				Sensory integration
taenia tecta	++	++	++	
frontal cortex	++	++	++	
orbital cortex	++	++	++	
agranular insular cortex	+++	++	++	
cingulate cortex	++	++	+	
retrosplenial cortex	++	++	+	
parietal cortex	++	++	++	Processing of visual stimuli
occipital cortex	++	++	++	
temporal cortex	++	++	++	Processing of auditory stimuli
perirhinal cortex	++	++		
entorhinal cortex	++	++	++	Processing of visceral information
dorsal endopiriform n	++	++	++	
piriform cortex	+++	+++	+++	Integration/ transmission of incoming olfactory information
Basal Ganglia				
accumbens n	+	++	++	Modulation of dopaminergic function
caudateputamen	+	+	++	Sensory/motor integration
globus pallidus	+	—	+	
Septum				
medial septum	++	++	+	Cognitive enhancement via cholinergic system

TABLE 3-continued

Distribution of rGABA$_B$R2, rGABA$_B$R1a, and GABA$_B$1b mRNA in the rat CNS. The strength of the hybridization signal for each of the respective mRNAs obtained in various regions of the rat brain was graded as weak (+), moderate (++), heavy (+++) or intense (++++) and is relative to the individual polypeptides.

Region	GABA$_B$R2	GABA$_B$R1a*	GABA$_B$R1b*	Potential Application
lateral septum	++	+	++	Modulation of integration of stimuli associated with adaptation
septohippo-campal n	+	+	+++	
diagonal band n	++	++	++	
ventral pallidum	++	+	+	
Amygdala				Anxiolytic (activation - reduction in panic attacks) appetite, depression
basolateral n	++	+	+	
medial amygdaloid n	+	+	+	Olfactory amygdala
basomedial n	+	+		
central n	+++	—	+	
anterior cortical n	+	+	+	
posteromedial cortical n	++	+	+	
bed n stria terminals	++	+	++	
zona incerta	+	+	+	
Hippocampus				Memory consolidation and retention
CA1 , Ammon's horn	++	+++	+++	
CA2 , Ammon's horn	++++	+++	+++	
CA3 , Ammon's horn	++++	+++	+++	Facilitation of LTP
subiculum	+	+++	+++	
parasubiculum	++	++	++	
presubiculum	++	++	++	
dentate gyrus	++++	+++	++	
polymorph dentate gyrus	+++	+++	++	
Hypothalamus				
suprachiasmatic n	+	++	ND	
median preoptic area	+	+	++	Regulation of gonadotropin secretion and reproductive behaviors
paraventricular n	+	++	++	Appetite/obesity
arcuate n	++	++	++	
anterior hypoth, post	+	+		
lateral hypoth	+	+	++	
ventromedial n	+	++	+++	
periventricular n	+	+	+	
supraoptic n	+	++	+	Synthesis of OXY and AVP
supramammillary n	++	++	++	Modulation of hypothalamic projections to cortex

TABLE 3-continued

Distribution of rGABA$_B$R2, rGABA$_B$R1a, and GABA$_B$1b mRNA in the rat CNS. The strength of the hybridization signal for each of the respective mRNAs obtained in various regions of the rat brain was graded as weak (+), moderate (++), heavy (+++) or intense (++++) and is relative to the individual polypeptides.

Region	GABA$_B$R2	GABA$_B$R1a*	GABA$_B$R1b*	Potential Application
premammillary n	+	+	+	
medial mammillary n	+	++	+	
Thalamus				Analgesia/Modulation of sensory information
paraventricular n	++	+	++	Modulation of motor and behavioral responses to pain
centromedial n	++	+	++	Modulation of motor and behavioral responses to pain
paracentral n.	++	+	++	
parafascicular n	++	+	++	Modulation of motor and behavioral responses to pain
anterodorsal n	+++	+	++	Modulation of eye movement
laterodorsal n	+++	+	++	
lateral posterior n	++	+	++	
reuniens n	+++	+	++	Modulation of thalamic input to ventral hippocampus and entorhinal ctx
rhomboid n	+++	+	++	
medial habenula	++++	+	++++	Anxiety/sleep disorders/ analgesia in chronic pain
lateral habenula	+	+	+++	
ventrolateral n	+++	+	++	
ventromedial n	+++	++	++	
ventral posterolateral n	+++	+	++	
reticular n	++	+	+	Alertness/sedation
lateral geniculate n	++	+	++	Modulation of visual perception
medial geniculate	++	+	++	Modulation of auditory system
subthalamic n	++	++	++	
Mesencephalon				
superior colliculus	+	+	+	Modulation of vision
inferior colliculus	+	+	+	
central gray	+	+	+	Analgesia
dorsal raphe	+	++	+	
deep mesencephalic n	+	+	+	
phalic n				
oculomotor n	+			
pontine n	+++		++	
retrorubral field	+			

TABLE 3-continued

Distribution of rGABA$_B$R2, rGABA$_B$R1a, and GABA$_B$1b mRNA in the rat CNS. The strength of the hybridization signal for each of the respective mRNAs obtained in various regions of the rat brain was graded as weak (+), moderate (++), heavy (+++) or intense (++++) and is relative to the individual polypeptides.

Region	GABA$_B$R2	GABA$_B$R1a*	GABA$_B$R1b*	Potential Application
ventral tegmental area	+	++	++	Modulation of the integration of motor behavior and adaptive responses
substantia nigra, reticular	+	+	+	Motor control
substantia nigra, compact	++	++	++	
interpedunc ular n	++	ND	ND	Analgesia
Myelencephalon				Analgesia
raphe magnus	++		++	
raphe pallidus	+	++	ND	
principal trigeminal	+	+		
spinal trigeminal n	+	+	+	
pontine reticular n	++	+	++	
parvicellular reticular n	+	++	++	
locus coeruleus	++	++	++	Modulation of NA transmission
parabrachial n	+	++	+	Modulation of visceral sensory information
vestibular n	+	++	+	Maintenance of balance and equilibrium
gigantocel l - ular reticular n	+	++	++	Inhibition and disinhibition of brainstem
prepositus hypoglossal n	+	+++	++	Position and movement of the eyes/ Modulation of arterial pressure and heart rate
ventral cochlear n	++	+	ND	
n soltary tract	++			Hypertension
A5 Noradrenaline cells	+	ND	ND	
facial n(7)	+	++	+	
Cerebellum				Motor coordination, Autism
granule cell layer	+	+	+	
Purkinje cells	++	—	++	
Spinal cord				Analgesia
dorsal horn	+	++	+	
ventral horn	+	++	+	
trigeminal ganglion	++	+++	+	Nociception
dorsal root ganglion	++++	+++	ND	Nociception

ND = not determined
*Bischoff S et al.

List of Abbreviations

[0441] 7 facial n

[0442] ac anterior commisure

[0443] Acb accumbens n

[0444] ACo anterior cortical amygdaloid n

[0445] AI agranular insular cortex

[0446] AON anterior olfactory n

[0447] APir amygdalopiriform transition area

[0448] APT anterior pretectal n

[0449] Arc arcuate hypothalamic n

[0450] BLA basolateral amygdaloid n

[0451] CA1-3 Fields of Ammon's horn

[0452] cc corpus callosum

[0453] Cg cingulate cortex

[0454] CeA central amygdaloid n

[0455] CPU caudate-putamen

[0456] DG dentate gyrus

[0457] DLG dorsal lateral geniculate n

[0458] DpMe deep mesencephalic n

[0459] Ent entorhinal cortex

[0460] Gi gigantocellular reticular n

[0461] Gr granule cll layer, cerebellum

[0462] GrO granule layer olf. bulb

[0463] FrA frontal association cortex

[0464] GP globus pallidus

[0465] HDB horizontal diagonal band

[0466] LA lateral amygdaloid n

[0467] LH lateral hypothalamus

[0468] LO lateral orbital cortex

[0469] LV lateral ventricle

[0470] Ml primary motor cortex

[0471] MeAD medial amygdaloid n, anterodorsal

[0472] MG medial geniculate

[0473] MHb medial habenular n

[0474] MPO medial preoptic n

[0475] PC Purkinje cell layer of the cerebellum

[0476] PF parafascicular n

[0477] Pir piriform cortex

[0478] PMCO posteromedial cortical amygdaloid n

[0479] Pr prepositus n

[0480] PVA paraventricular thalamic n

[0481] RS retrosplenial cortex

[0482] S subiculum

[0483] SFi septofimbrial n

[0484] SI substantia innominata

[0485] SNc substantia nigra,compact

[0486] STh subthalamic n

[0487] Sp5 spinal trigeminal n

[0488] TT tenia tecta

[0489] Ve vestibular n

[0490] VTA ventral tegmental area

Potential Therapeutic Application for $GABA_B$ Agonists and Antagonists

Agonists

Antinociception

[0491] A potential $GABA_B$ agonist application may in antinociception. The inhibitory effects of GABA and $GABA_B$ agonists are thought to be predominantly a presynaptic mechanism on excitation-induced impulses in high threshold Ad and C fibers on primary afferents. This effect can be blocked by $GABA_B$ antagonists (Hao,J- H., et al., 1994). Baclofen's spinal cord analgesic effects have been well documented in the rat, though it has not been as effective in human. However, baclofen has been successful in the treatment of trigeminal neuralgia in human.

[0492] The localization of the $GABA_BR2$ mRNA in the superficial layers of the spinal cord dorsal horn, the termination site for primary afferents, as well as their cells of origin in the dorsal root and trigeminal ganglia position the $GABA_BR1/R2$ receptor appropriately for mediating the agonist effects.

Drug Addiction

[0493] It has been suggested that GABA agonists may have some potential in the treatment of cocaine addiction. A role for the action of psychostimulants in the mesoaccumbens dopamine system is well established. The ventral pallidum receives a GABAergic projection from the nucleus accumbens and both regions contain $GABA_BR2$ transcripts. GABA receptors were shown to have an inhibitory effect on dopamine release in the ventral pallidum. Phaclofen acting at these receptors resulted in increased dopamine release and baclofen was shown to attenuate the reinforcing effects of cocaine. (Roberts, D. C. S., et al.,1996; Morgan,A. E. et al.)

Micturition

[0494] There is a potential application for $GABA_B$ agonists in the treatment of bladder dysfunction. Baclofen has been used in the treatment of detrussor hyperreflexia through inhibition of contractile responses. In addition to a peripheral site of action for $GABA_B$ agonists, there is also the possibility for a central site. The pontine micturition center in the brainstem is involved in mediating the spinal reflex pathway, via Onuf's nucleus in the sacral spinal cord. Support for possible application of $GABA_B$ agonists in the treatment of bladder dysfunction may be augmented by presence of $GABA_BR2$ mRNA in the various nuclei involved in the control of the lower urinary tract function.

Antagonists

Memory Enhancement—Alzheimer's Disease

[0495] $GABA_B$ antagonists may have a potential application in the treatment of Alzheimer's Disease. The blockade of $GABA_B$ receptors might lead to signal amplification and improvement in cognitive functions resulting from an increased excitability of cortical neurons via amplification of the acetycholine signal. Additionally, memory may be enhanced by $GABA_B$ antagonists which have been shown to suppress late IPSPs, thus facilitating long-term potentiation in the hippocampus (see Table 3).

[0496] To support this idea, CGP36742, a $GABA_B$ antagonist, has been shown to improve learning performance in aged rats as well as the performance of rhesus monkeys in conditioned spatial color task. (Mondadori, C. et al., 1993). The significance of the $GABA_BR1/R2$ receptor in cognitive functioning might be indicated by the presence of $GABA_BR2$ mRNA in the cerebral cortex and its codistribution in the ventral forebrain with cortically projecting cholinergic neurons as well as its localization in the pyramidal cells in all regions of Ammon's horn and dentate gyrus in the hippocampus.

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aacaacaagc ggtcaggcgt ggggcccagc aagttccacg ggtacgccta cgatggcatc  1200
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cagcggatcc aggacttcaa ctacacggac cacacgctgg gcaggatcat cctcaatgcc  1320
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atcatccttg gagggatgct ttcctatgct tccatatttc tctttggcct tgatggatcc  1740
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aaaaatgtga aaatgaagaa gaagatcatc aaggaccaga aactgcttgt gatcgtgggg  1920
ggcatgctgc tgatcgacct gtgtatcctg atctgctggc aggctgtgga ccccctgcga  1980
aggacagtgg agaagtacag catggagccg gacccagcag gacgggatat ctccatccgc  2040
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tacaagggac ttctcatgtt gttcggttgt ttcttagctt gggagacccg caacgtcagc  2160
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atcgtggctc tggtcatcat cttctgcagc accatcaccc tctgcctggt attcgtgccg  2340
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actcagaatc agaagaaaga agattctaaa acgtccacct cggtcaccag tgtgaaccaa  2460
gccagcacat cccgcctgga gggcctacag tcagaaaacc atcgcctgcg aatgaagatc  2520
acagagctgg ataaagactt ggaagaggtc accatgcagc tgcaggacac accagaaaag  2580
accacctaca ttaaacagaa ccactaccaa gagctcaatg acatcctcaa cctgggaaac  2640
ttcactgaga gcacagatgg aggaaaggcc attttaaaaa atcacctcga tcaaaatccc  2700
cagctacagt ggaacacaac agagccctct cgaacatgca aagatcctat agaagatata  2760
aactctccag aacacatcca gcgtcggctg tccctccagc tccccatcct ccaccacgcc  2820
tacctcccat ccatcggagg cgtggacgcc agctgtgtca gcccctgcgt cagccccacc  2880
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gccagccccc gccacagaca tgtgccaccc tccttccgag tcatggtctc gggcctgtaa 2940

gggtgggagg cctgggcccg gggcctcccc cgtgacagaa ccacactggg cagaggggtc 3000

tgctgcagaa acactgtcgg ctctggctgc ggagaagctg ggcaccatgg ctggcctctc 3060

aggaccactc ggatggcact caggtggaca ggacggggca gggggagact tggcacctga 3120

cctcgagcct tatttgtgaa gtccttattt cttcacaaag aagaggaacg gaaatgggac 3180

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aaaa 3244

<210> SEQ ID NO 2
<211> LENGTH: 898
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 2

Met Pro Ser Cys Pro Ala Arg Ser Ala Thr Gly Pro Leu Ser Ile Met
1 5 10 15

Gly Leu Met Pro Leu Thr Lys Glu Val Ala Lys Gly Ser Ile Gly Arg
20 25 30

Gly Val Leu Pro Ala Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu
35 40 45

Ser Leu Leu Arg Pro Tyr Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu
50 55 60

Cys Asp Asn Ala Lys Gly Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr
65 70 75 80

Gly Pro Asn His Leu Met Val Phe Gly Gly Val Cys Pro Ser Val Thr
85 90 95

Ser Ile Ile Ala Glu Ser Leu Gln Gly Trp Asn Leu Val Gln Leu Ser
100 105 110

Phe Ala Ala Thr Thr Pro Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr
115 120 125

Phe Phe Arg Thr Val Pro Ser Asp Asn Ala Val Asn Pro Ala Ile Leu
130 135 140

Lys Leu Leu Lys His Tyr Gln Trp Lys Arg Val Gly Thr Leu Thr Gln
145 150 155 160

Asp Val Gln Arg Phe Ser Glu Val Arg Asn Asp Leu Thr Gly Val Leu
165 170 175

Tyr Gly Glu Asp Ile Glu Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp
180 185 190

Pro Cys Thr Ser Val Lys Lys Leu Lys Gly Asn Asp Val Arg Ile Ile
195 200 205

Leu Gly Gln Phe Asp Gln Asn Met Ala Ala Lys Val Phe Cys Cys Ala
210 215 220

Tyr Glu Glu Asn Met Tyr Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly
225 230 235 240

Trp Tyr Glu Pro Ser Trp Trp Glu Gln Val His Thr Glu Ala Asn Ser
245 250 255

Ser Arg Cys Leu Arg Lys Asn Leu Leu Ala Ala Met Glu Gly Tyr Ile
260 265 270

Gly Val Asp Phe Glu Pro Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser
275 280 285

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Gly Lys Thr Pro Gln Gln Tyr Glu Arg Glu Tyr Asn Asn Lys Arg Ser
    290                 295                 300

Gly Val Gly Pro Ser Lys Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp
305                 310                 315                 320

Val Ile Ala Lys Thr Leu Gln Arg Ala Met Glu Thr Leu His Ala Ser
                325                 330                 335

Ser Arg His Gln Arg Ile Gln Asp Phe Asn Tyr Thr Asp His Thr Leu
            340                 345                 350

Gly Arg Ile Ile Leu Asn Ala Met Asn Glu Thr Asn Phe Phe Gly Val
        355                 360                 365

Thr Gly Gln Val Val Phe Arg Asn Gly Glu Arg Met Gly Thr Ile Lys
    370                 375                 380

Phe Thr Gln Phe Gln Asp Ser Arg Glu Val Lys Val Gly Glu Tyr Asn
385                 390                 395                 400

Ala Val Ala Asp Thr Leu Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln
                405                 410                 415

Gly Ser Glu Pro Pro Lys Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg
            420                 425                 430

Lys Ile Ser Leu Pro Leu Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu
        435                 440                 445

Gly Met Ile Met Ala Ser Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg
    450                 455                 460

Asn Gln Lys Leu Ile Lys Met Ser Ser Pro Tyr Met Asn Asn Leu Ile
465                 470                 475                 480

Ile Leu Gly Gly Met Leu Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu
                485                 490                 495

Asp Gly Ser Phe Val Ser Glu Lys Thr Phe Glu Thr Leu Cys Thr Val
            500                 505                 510

Arg Thr Trp Ile Leu Thr Val Gly Tyr Thr Thr Ala Phe Gly Ala Met
        515                 520                 525

Phe Ala Lys Thr Trp Arg Val His Ala Ile Phe Lys Asn Val Lys Met
    530                 535                 540

Lys Lys Lys Ile Ile Lys Asp Gln Lys Leu Leu Val Ile Val Gly Gly
545                 550                 555                 560

Met Leu Leu Ile Asp Leu Cys Ile Leu Ile Cys Trp Gln Ala Val Asp
                565                 570                 575

Pro Leu Arg Arg Thr Val Glu Lys Tyr Ser Met Glu Pro Asp Pro Ala
            580                 585                 590

Gly Arg Asp Ile Ser Ile Arg Pro Leu Leu Glu His Cys Glu Asn Thr
        595                 600                 605

His Met Thr Ile Trp Leu Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu
    610                 615                 620

Met Leu Phe Gly Cys Phe Leu Ala Trp Glu Thr Arg Asn Val Ser Ile
625                 630                 635                 640

Pro Ala Leu Asn Asp Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn Val
                645                 650                 655

Gly Ile Met Cys Ile Ile Gly Ala Ala Val Ser Phe Leu Thr Arg Asp
            660                 665                 670

Gln Pro Asn Val Gln Phe Cys Ile Val Ala Leu Val Ile Ile Phe Cys
        675                 680                 685

Ser Thr Ile Thr Leu Cys Leu Val Phe Val Pro Lys Leu Ile Thr Leu
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    690                 695                 700

Arg Thr Asn Pro Asp Ala Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr
705                 710                 715                 720

Gln Asn Gln Lys Lys Glu Asp Ser Lys Thr Ser Thr Ser Val Thr Ser
                725                 730                 735

Val Asn Gln Ala Ser Thr Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn
            740                 745                 750

His Arg Leu Arg Met Lys Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu
        755                 760                 765

Val Thr Met Gln Leu Gln Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys
    770                 775                 780

Gln Asn His Tyr Gln Glu Leu Asn Asp Ile Leu Asn Leu Gly Asn Phe
785                 790                 795                 800

Thr Glu Ser Thr Asp Gly Gly Lys Ala Ile Leu Lys Asn His Leu Asp
                805                 810                 815

Gln Asn Pro Gln Leu Gln Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys
            820                 825                 830

Lys Asp Pro Ile Glu Asp Ile Asn Ser Pro Glu His Ile Gln Arg Arg
        835                 840                 845

Leu Ser Leu Gln Leu Pro Ile Leu His His Ala Tyr Leu Pro Ser Ile
    850                 855                 860

Gly Gly Val Asp Ala Ser Cys Val Ser Pro Cys Val Ser Pro Thr Ala
865                 870                 875                 880

Ser Pro Arg His Arg His Val Pro Pro Ser Phe Arg Val Met Val Ser
                885                 890                 895

Gly Leu
```

<210> SEQ ID NO 3
<211> LENGTH: 2823
<212> TYPE: DNA
<213> ORGANISM: Rattus Sp.

<400> SEQUENCE: 3

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cgcctgctgc tgcccctgct gctgtcgctg ctgctgtggt tggcgcccgg ggcctggggc    120

tggacgcggg gcgccccccg gccgccgccc agcagcccgc cgctctccat catgggcctc    180

atgccgctca ccaaggaggt ggccaagggc agcatcgggc gcggcgtgct ccccgccgtg    240

gagctagcca tcgagcagat ccgcaacgag tcactcctgc gcccctactt cctggacctg    300

cgactctatg acaccgagtg tgacaatgca aagggactga aagccttcta tgacgcaata    360

aagtatgggc cgaaccattt gatggtgttt ggaggcgtct gtccgtctgt cacatctatt    420

atcgcggagt ccctccaagg ctggaatctg gtgcagcttt ccttcgccgc caccacgcct    480

gttcttgcgg ataagaagaa gtacccgtat ttcttccgga cggtgccgtc agacaacgcg    540

gtgaaccccg ccatcctgaa gctcctgaag cacttccgct ggcggcgtgt gggcacactc    600

acgcaggacg tgcagcgctt ctccgaggtg aggaatgacc tgactggggt tctgtatggg    660

gaagatattg agatctcaga cacagagagt ttctccaatg atccctgcac cagcgtcaaa    720

aagctcaagg ggaatgacgt gcggatcatc cttggccagt ttgaccagaa tatggcagca    780

aaagtcttct gttgtgcctt cgaggagagc atgtttggca gcaagtacca gtggatcatc    840
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ccgggatggt acgagcctgc gtggtgggag caggtgcatg tggaggccaa ttcctcacgc    900
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ctgagctcca aacaaatcaa gaccatctca gggaagactc cacagcagta tgaaagagag   1020
tacaacagca aacgttcagg cgtggggccc agcaagttcc atgggtacgc ctacgatggg   1080
atctgggtca tcgccaagac cctacagagg gccatggaga cactgcatgc cagtagcagg   1140
caccagcgga tccaggactt caactacaca gaccacacgc tgggcaaaat catcctcaat   1200
gccatgaacg agaccaactt cttcggggtc acgggtcaag ttgtgttccg gaacggggag   1260
agaatgggaa ccattaaatt tactcaattt caagacagca gagaggtgaa ggtcggcgaa   1320
tacaacgcgg tggctgacac actggagatc atcaatgaca ccataaggtt ccaggggtcc   1380
gagccaccca aggacaagac catcattctg gagcagcttc ggaagatctc gcttccactg   1440
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ttcaacatca agaaccggaa ccaaaagctg attaagatgt caagccccta catgaacaac   1560
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gtgggctaca caactgcctt tggggccatg tttgcaaaga cctggagggt ccatgccatc   1740
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gggggcatgc tgctcatcga cctgtgcatc ctgatctgtt ggcaggctgt ggaccccctg   1860
cggaggacag tagagaggta cagcatggag ccggacccag caggccggga catctccatc   1920
cgcccattgc tggaacactg cgaaaacacc cacatgacca tctggcttgg cattgtctac   1980
gcctacaagg ggctcctcat gctattcggt tgtttcttgg catgggaaac ccgcaatgtg   2040
agcatccctg ccctcaacga cagcaagtac atcggcatga gtgtgtacaa tgtggggatc   2100
atgtgcatca tcggggctgc tgtctccttc ctgacgcgtg accagcccaa cgtgcagttc   2160
tgcatcgtgg ccctggtcat catcttctgc agcaccatca ctctctgcct ggtgtttgtg   2220
ccaaagctca ttactctgag gacaaaccct gacgcagcca ctcagaacag gcggttccag   2280
ttcacacaga accagaagaa agaagattcg aagacctcca cttcagtcac cagcgtgaac   2340
caggcgagca cgtcacgcct ggagggactg cagtcagaaa accaccgcct tcgaatgaag   2400
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aagaccacat acatcaaaca gaatcactac caagagctca acgacatcct cagcttgggc   2520
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ccccagctcc agtggaacac gacagagccc tcaagaacat gcaaagaccc catagaagac   2640
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gcctacctcc catccatcgg aggcgtggat gccagctgcg tcagcccctg tgtcagccct   2760
accgccagcc ctcgccacag acacgtacca ccctccttcc gagtcatggt ctcgggcctg   2820
tag                                                                 2823
```

<210> SEQ ID NO 4
<211> LENGTH: 929
<212> TYPE: PRT
<213> ORGANISM: Rattus Sp.

<400> SEQUENCE: 4

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Met Ala Ser Pro Pro Ser Ser Gly Gln Pro Arg Pro Pro Pro Pro Pro
  1               5                  10                  15

Pro Pro Pro Ala Arg Leu Leu Leu Pro Leu Leu Leu Ser Leu Leu Leu
             20                  25                  30

Trp Leu Ala Pro Gly Ala Trp Gly Trp Thr Arg Gly Ala Pro Arg Pro
         35                  40                  45

Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu Thr
     50                  55                  60

Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala Val
 65                  70                  75                  80

Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro Tyr
                 85                  90                  95

Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys Gly
            100                 105                 110

Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu Met
        115                 120                 125

Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu Ser
    130                 135                 140

Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr Pro
145                 150                 155                 160

Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val Pro
                165                 170                 175

Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His Phe
            180                 185                 190

Arg Trp Arg Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe Ser
        195                 200                 205

Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile Glu
    210                 215                 220

Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val Lys
225                 230                 235                 240

Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp Gln
                245                 250                 255

Asn Met Ala Ala Lys Val Phe Cys Cys Ala Phe Glu Glu Ser Met Phe
            260                 265                 270

Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ala Trp
        275                 280                 285

Trp Glu Gln Val His Val Glu Ala Asn Ser Ser Arg Cys Leu Arg Arg
    290                 295                 300

Ser Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu Pro
305                 310                 315                 320

Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln Gln
                325                 330                 335

Tyr Glu Arg Glu Tyr Asn Ser Lys Arg Ser Gly Val Gly Pro Ser Lys
            340                 345                 350

Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr Leu
        355                 360                 365

Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg Ile
    370                 375                 380

Lys Ile Ile Leu Asn Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr
385                 390                 395                 400

Gly Gln Val Val Phe Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe
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                405                 410                 415

Thr Gln Phe Gln Asp Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala
            420                 425                 430

Val Ala Asp Thr Leu Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly
        435                 440                 445

Ser Glu Pro Pro Lys Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys
    450                 455                 460

Ile Ser Leu Pro Leu Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly
465                 470                 475                 480

Met Ile Met Ala Ser Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn
                485                 490                 495

Gln Lys Leu Ile Lys Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile
            500                 505                 510

Leu Gly Gly Met Leu Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp
        515                 520                 525

Gly Ser Phe Val Ser Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg
    530                 535                 540

Thr Trp Ile Leu Thr Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe
545                 550                 555                 560

Ala Lys Thr Trp Arg Val His Ala Ile Phe Lys Asn Val Lys Met Lys
                565                 570                 575

Lys Lys Ile Ile Lys Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met
            580                 585                 590

Leu Leu Ile Asp Leu Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro
        595                 600                 605

Leu Arg Arg Thr Val Glu Arg Tyr Ser Met Glu Pro Asp Pro Ala Gly
    610                 615                 620

Arg Asp Ile Ser Ile Arg Pro Leu Leu Glu His Cys Glu Asn Thr His
625                 630                 635                 640

Met Thr Ile Trp Leu Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met
                645                 650                 655

Leu Phe Gly Cys Phe Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro
            660                 665                 670

Ala Leu Asn Asp Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly
        675                 680                 685

Ile Met Cys Ile Ile Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln
    690                 695                 700

Pro Asn Val Gln Phe Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser
705                 710                 715                 720

Thr Ile Thr Leu Cys Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg
                725                 730                 735

Thr Asn Pro Asp Ala Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln
            740                 745                 750

Asn Gln Lys Lys Glu Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val
        755                 760                 765

Asn Gln Ala Ser Thr Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His
    770                 775                 780

Arg Leu Arg Met Lys Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val
785                 790                 795                 800

Thr Met Gln Leu Gln Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln
                805                 810                 815
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```
Asn His Tyr Gln Glu Leu Asn Asp Ile Leu Ser Leu Gly Asn Phe Thr
            820                 825                 830

Glu Ser Thr Asp Gly Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln
        835                 840                 845

Asn Pro Gln Leu Gln Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys
    850                 855                 860

Asp Pro Ile Glu Asp Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu
865                 870                 875                 880

Ser Leu Gln Leu Pro Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly
                885                 890                 895

Gly Val Asp Ala Ser Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser
            900                 905                 910

Pro Arg His Arg His Val Pro Pro Ser Phe Arg Val Met Val Ser Gly
        915                 920                 925

Leu
```

<210> SEQ ID NO 5
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Oligonucleotide Probe

<400> SEQUENCE: 5

agggatgctt tcctatgctt ccatatttct ctttggcctt gatgg 45

<210> SEQ ID NO 6
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Oligonucleotide Probe

<400> SEQUENCE: 6

caatgtgcag ttctgcatcg tggctctggt catcatcttc tgcag 45

<210> SEQ ID NO 7
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 7

cttctaggcc tgtacggaag tgtt 24

<210> SEQ ID NO 8
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 8

gttgtggttt gtccaaactc atcaat 26

<210> SEQ ID NO 9

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<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 9

gggatgagtg tctacaacgt gggg 24

<210> SEQ ID NO 10
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 10

tgcgttgctg catctgggtt tgttct 26

<210> SEQ ID NO 11
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 11

atctccctac ctctctacag catcct 26

<210> SEQ ID NO 12
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 12

caggtcctga cggtgcaaag tgtttc 26

<210> SEQ ID NO 13
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 13

tgacgcaaga cgttcagagg ttctct 26

<210> SEQ ID NO 14
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 14

tgtagccttc catggcagca agcaga 26

<210> SEQ ID NO 15
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 15

agagaacctc tgaacgtctt gcgtca 26

<210> SEQ ID NO 16
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 16

ggctctgttg tgttccactg tagctg 26

<210> SEQ ID NO 17
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 17

tcatgccgct caccaaggag gtggcc 26

<210> SEQ ID NO 18
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 18

ggccacctcc ttggtgagcg gcatga 26

<210> SEQ ID NO 19
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 19

tgagtgagca gagtccagag ccgt 24

<210> SEQ ID NO 20
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 20

atggatggga ggtaggcgtg gtggag 26

<210> SEQ ID NO 21
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 21

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ctctctgccc tcaccatcct cgggat 26

<210> SEQ ID NO 22
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 22

gactccggct cgaataccag gcagag 26

<210> SEQ ID NO 23
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 23

ccatgtttgc aaagacctgg agggtcc 27

<210> SEQ ID NO 24
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 24

ggtcacgcgt caggaaagag acagcag 27

<210> SEQ ID NO 25
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 25

aagcttctag agatcccctcg acctc 25

<210> SEQ ID NO 26
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 26

aggcgcagaa ctggtaggta tggaa 25

<210> SEQ ID NO 27
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 27

cttctaggcc tgtacggaag tgtta 25

<210> SEQ ID NO 28

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<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 28

gttgtggttt gtccaaactc atcaatg 27

<210> SEQ ID NO 29
<211> LENGTH: 27
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 29

ctgctgtctc tttcctgacg cgtgacc 27

<210> SEQ ID NO 30
<211> LENGTH: 59
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 30

ccaagcttct aatacgactc actatagggg agaccatggg cccgggggga ccctgtacc 59

<210> SEQ ID NO 31
<211> LENGTH: 63
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 31

ttttttttt ttttttttt ttttttttt ttttcactt gtaaagcaaa tgtactcgac 60

tcc 63

<210> SEQ ID NO 32
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 32

cgcggatcca ttatgtctgc actccgaagg aaatttg 37

<210> SEQ ID NO 33
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 33

cgcgaattct tatgtgaagc gatcagagtt catttttc 38

<210> SEQ ID NO 34
<211> LENGTH: 34
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 34

gcgggatccg ctatggctgg tgattctagg aatg 34

<210> SEQ ID NO 35
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Primer

<400> SEQUENCE: 35

ccggaattcc cctcacaccg agcccctgg 29

<210> SEQ ID NO 36
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide Probe

<400> SEQUENCE: 36

gcaataaagt atgggctgaa ccatttgatg gtgtttggag gcgt 44

<210> SEQ ID NO 37
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide Probe

<400> SEQUENCE: 37

acgcctccaa acaccatcaa atggttcagc ccatacttta ttgc 44

<210> SEQ ID NO 38
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide Probe

<400> SEQUENCE: 38

tttgagcccc tgagctccaa acaaatcaag accatctcag 40

<210> SEQ ID NO 39
<211> LENGTH: 40
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide Probe

<400> SEQUENCE: 39

ctgagatggt cttgatttgt ttggagctca ggggctcaaa 40

<210> SEQ ID NO 40
<211> LENGTH: 43
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide Probe

<400> SEQUENCE: 40

aaggccatca acttcctgcc tgtggactat gagatcgaat atg 43

<210> SEQ ID NO 41
<211> LENGTH: 43
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide Probe

<400> SEQUENCE: 41

catattcgat ctcatagtcc acaggcagga agttgatggc ctt 43

<210> SEQ ID NO 42
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide Probe

<400> SEQUENCE: 42

tggccgctgc ctcttctgct ggtgatggcg gctggggt 38

<210> SEQ ID NO 43
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide Probe

<400> SEQUENCE: 43

accccagccg ccatcaccag cagaagaggc agcggcca 38

<210> SEQ ID NO 44
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide Probe

<400> SEQUENCE: 44

ccttggcttt ggccttgaac aagacgtctg gaggaggtgg tcgtt 45

<210> SEQ ID NO 45
<211> LENGTH: 45
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence:
Oligonucleotide Probe

<400> SEQUENCE: 45

aacgaccacc tcctccagac gtcttgttca aggccaaagc caagg 45

<210> SEQ ID NO 46

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<211> LENGTH: 2826
<212> TYPE: DNA
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 46

atggcttccc cgcggagctc cgggcagccc gggccgccgc cgccgccgcc accgccgccc 60

gcgcgcctgc tactgctact gctgctgccg ctgctgctgc ctctggcgcc cggggcctgg 120

ggctgggcgc ggggcgcccc ccggccgccg cccagcagcc cgccgctctc catcatgggc 180

ctcatgccgc tcaccaagga ggtggccaag ggcagcatcg ggcgcggtgt gctccccgcc 240

gtggaactgg ccatcgagca gatccgcaac gagtcactcc tgcgccccta cttcctcgac 300

ctgcggctct atgacacgga gtgcgacaac gcaaaagggt tgaaagcctt ctacgatgcg 360

ataaaatacg ggccgaacca cttgatggtg tttggaggcg tctgtccatc cgtcacatcc 420

atcattgcag agtccctcca aggctggaat ctggtgcagc tttcttttgc tgcaaccacg 480

cctgttctag ccgataagaa aaaataccct tatttctttc ggaccgtccc atcagacaat 540

gcggtgaatc cagccattct gaagttgctc aagcactacc agtggaagcg cgtgggcacg 600

ctgacgcaag acgttcagag gttctctgag gtgcggaatg acctgactgg agttctgtat 660

ggcgaggaca ttgagatttc agacaccgag agcttctcca acgatccctg taccagtgtc 720

aaaaagctga aggggaatga tgtgcggatc atccttggcc agtttgacca gaatatggca 780

gcaaaagtgt tctgttgtgc atacgaggag aacatgtatg gtagtaaata tcagtggatc 840

attccgggct ggtacgagcc ttcttggtgg gagcaggtgc acacggaagc caactcatcc 900

cgctgcctcc ggaagaatct gcttgctgcc atggagggct acattggcgt ggatttcgag 960

cccctgagct ccaagcagat caagaccatc tcaggaaaga ctccacagca gtatgagaga 1020

gagtacaaca acaagcggtc aggcgtgggg cccagcaagt tccacgggta cgcctacgat 1080

ggcatctggg tcatcgccaa gacactgcag agggccatgg agacactgca tgccagcagc 1140

cggcaccagc ggatccagga cttcaactac acggaccaca cgctgggcag gatcatcctc 1200

aatgccatga acgagaccaa cttcttcggg gtcacgggtc aagttgtatt ccggaatggg 1260

gagagaatgg ggaccattaa atttactcaa tttcaagaca gcagggaggt gaaggtggga 1320

gagtacaacg ctgtggccga cacactggag atcatcaatg acaccatcag gttccaagga 1380

tccgaaccac caaaagacaa gaccatcatc ctggagcagc tgcggaagat ctccctacct 1440

ctctacagca tcctctctgc cctcaccatc ctcgggatga tcatggccag tgcttttctc 1500

ttcttcaaca tcaagaaccg gaatcagaag ctcataaaga tgtcgagtcc atacatgaac 1560

aaccttatca tccttggagg gatgctttcc tatgcttcca tatttctctt tggccttgat 1620

ggatcctttg tctctgaaaa gacctttgaa acactttgca ccgtcaggac ctggattctc 1680

accgtgggct acacgaccgc ttttggggcc atgtttgcaa agacctggag agtccacgcc 1740

atcttcaaaa atgtgaaaat gaagaagaag atcatcaagg accagaaact gcttgtgatc 1800

gtgggggggca tgctgctgat cgacctgtgt atcctgatct gctggcaggc tgtggacccc 1860

ctgcgaagga cagtggagaa gtacagcatg gagccggacc cagcaggacg ggatatctcc 1920

atccgccctc tcctggagca ctgtgagaac acccatatga ccatctggct tggcatcgtc 1980

tatgcctaca agggacttct catgttgttc ggttgtttct tagcttggga gacccgcaac 2040

gtcagcatcc ccgcactcaa cgacagcaag tacatcggga tgagtgtcta caacgtgggg 2100

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atcatgtgca tcatcggggc cgctgtctcc ttcctgaccc gggaccagcc caatgtgcag   2160

ttctgcatcg tggctctggt catcatcttc tgcagcacca tcaccctctg cctggtattc   2220

gtgccgaagc tcatcaccct gagaacaaac ccagatgcag caacgcagaa caggcgattc   2280

cagttcactc agaatcagaa gaaagaagat tctaaaacgt ccacctcggt caccagtgtg   2340

aaccaagcca gcacatcccg cctggagggc ctacagtcag aaaaccatcg cctgcgaatg   2400

aagatcacag agctggataa agacttggaa gaggtcacca tgcagctgca ggacacacca   2460

gaaaagacca cctacattaa acagaaccac taccaagagc tcaatgacat cctcaacctg   2520

ggaaacttca ctgagagcac agatggagga aaggccattt taaaaaatca cctcgatcaa   2580

aatccccagc tacagtggaa cacaacagag ccctctcgaa catgcaaaga tcctatagaa   2640

gatataaact ctccagaaca catccagcgt cggctgtccc tccagctccc catcctccac   2700

cacgcctacc tcccatccat cggaggcgtg gacgccagct gtgtcagccc ctgcgtcagc   2760

cccaccgcca gcccccgcca cagacatgtg ccaccctcct tccgagtcat ggtctcgggc   2820

ctgtaa                                                              2826
```

<210> SEQ ID NO 47
<211> LENGTH: 941
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 47

```
Met Ala Ser Pro Arg Ser Ser Gly Gln Pro Gly Pro Pro Pro Pro Pro
  1               5                  10                  15

Pro Pro Pro Pro Ala Arg Leu Leu Leu Leu Leu Leu Leu Pro Leu Leu
             20                  25                  30

Leu Pro Leu Ala Pro Gly Ala Trp Gly Trp Ala Arg Gly Ala Pro Arg
         35                  40                  45

Pro Pro Pro Ser Ser Pro Pro Leu Ser Ile Met Gly Leu Met Pro Leu
     50                  55                  60

Thr Lys Glu Val Ala Lys Gly Ser Ile Gly Arg Gly Val Leu Pro Ala
 65                  70                  75                  80

Val Glu Leu Ala Ile Glu Gln Ile Arg Asn Glu Ser Leu Leu Arg Pro
                 85                  90                  95

Tyr Phe Leu Asp Leu Arg Leu Tyr Asp Thr Glu Cys Asp Asn Ala Lys
            100                 105                 110

Gly Leu Lys Ala Phe Tyr Asp Ala Ile Lys Tyr Gly Pro Asn His Leu
        115                 120                 125

Met Val Phe Gly Gly Val Cys Pro Ser Val Thr Ser Ile Ile Ala Glu
    130                 135                 140

Ser Leu Gln Gly Trp Asn Leu Val Gln Leu Ser Phe Ala Ala Thr Thr
145                 150                 155                 160

Pro Val Leu Ala Asp Lys Lys Lys Tyr Pro Tyr Phe Phe Arg Thr Val
                165                 170                 175

Pro Ser Asp Asn Ala Val Asn Pro Ala Ile Leu Lys Leu Leu Lys His
            180                 185                 190

Tyr Gln Trp Lys Arg Val Gly Thr Leu Thr Gln Asp Val Gln Arg Phe
        195                 200                 205

Ser Glu Val Arg Asn Asp Leu Thr Gly Val Leu Tyr Gly Glu Asp Ile
    210                 215                 220
```

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```
Glu Ile Ser Asp Thr Glu Ser Phe Ser Asn Asp Pro Cys Thr Ser Val
225                 230                 235                 240

Lys Lys Leu Lys Gly Asn Asp Val Arg Ile Ile Leu Gly Gln Phe Asp
                245                 250                 255

Gln Asn Met Ala Ala Lys Val Phe Cys Cys Ala Tyr Glu Glu Asn Met
            260                 265                 270

Tyr Gly Ser Lys Tyr Gln Trp Ile Ile Pro Gly Trp Tyr Glu Pro Ser
        275                 280                 285

Trp Trp Glu Gln Val His Thr Glu Ala Asn Ser Ser Arg Cys Leu Arg
    290                 295                 300

Lys Asn Leu Leu Ala Ala Met Glu Gly Tyr Ile Gly Val Asp Phe Glu
305                 310                 315                 320

Pro Leu Ser Ser Lys Gln Ile Lys Thr Ile Ser Gly Lys Thr Pro Gln
                325                 330                 335

Gln Tyr Glu Arg Glu Tyr Asn Asn Lys Arg Ser Gly Val Gly Pro Ser
            340                 345                 350

Lys Phe His Gly Tyr Ala Tyr Asp Gly Ile Trp Val Ile Ala Lys Thr
        355                 360                 365

Leu Gln Arg Ala Met Glu Thr Leu His Ala Ser Ser Arg His Gln Arg
    370                 375                 380

Ile Gln Asp Phe Asn Tyr Thr Asp His Thr Leu Gly Arg Ile Ile Leu
385                 390                 395                 400

Asn Ala Met Asn Glu Thr Asn Phe Phe Gly Val Thr Gly Gln Val Val
                405                 410                 415

Phe Arg Asn Gly Glu Arg Met Gly Thr Ile Lys Phe Thr Gln Phe Gln
            420                 425                 430

Asp Ser Arg Glu Val Lys Val Gly Glu Tyr Asn Ala Val Ala Asp Thr
        435                 440                 445

Leu Glu Ile Ile Asn Asp Thr Ile Arg Phe Gln Gly Ser Glu Pro Pro
    450                 455                 460

Lys Asp Lys Thr Ile Ile Leu Glu Gln Leu Arg Lys Ile Ser Leu Pro
465                 470                 475                 480

Leu Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met Ala
                485                 490                 495

Ser Ala Phe Leu Phe Phe Asn Ile Lys Asn Arg Asn Gln Lys Leu Ile
            500                 505                 510

Lys Met Ser Ser Pro Tyr Met Asn Asn Leu Ile Ile Leu Gly Gly Met
        515                 520                 525

Leu Ser Tyr Ala Ser Ile Phe Leu Phe Gly Leu Asp Gly Ser Phe Val
    530                 535                 540

Ser Glu Lys Thr Phe Glu Thr Leu Cys Thr Val Arg Thr Trp Ile Leu
545                 550                 555                 560

Thr Val Gly Tyr Thr Thr Ala Phe Gly Ala Met Phe Ala Lys Thr Trp
                565                 570                 575

Arg Val His Ala Ile Phe Lys Asn Val Lys Met Lys Lys Lys Ile Ile
            580                 585                 590

Lys Asp Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp
        595                 600                 605

Leu Cys Ile Leu Ile Cys Trp Gln Ala Val Asp Pro Leu Arg Arg Thr
    610                 615                 620

Val Glu Lys Tyr Ser Met Glu Pro Asp Pro Ala Gly Arg Asp Ile Ser
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625                 630                 635                 640

Ile Arg Pro Leu Leu Glu His Cys Glu Asn Thr His Met Thr Ile Trp
                645                 650                 655

Leu Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met Leu Phe Gly Cys
            660                 665                 670

Phe Leu Ala Trp Glu Thr Arg Asn Val Ser Ile Pro Ala Leu Asn Asp
        675                 680                 685

Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly Ile Met Cys Ile
    690                 695                 700

Ile Gly Ala Ala Val Ser Phe Leu Thr Arg Asp Gln Pro Asn Val Gln
705                 710                 715                 720

Phe Cys Ile Val Ala Leu Val Ile Ile Phe Cys Ser Thr Ile Thr Leu
                725                 730                 735

Cys Leu Val Phe Val Pro Lys Leu Ile Thr Leu Arg Thr Asn Pro Asp
            740                 745                 750

Ala Ala Thr Gln Asn Arg Arg Phe Gln Phe Thr Gln Asn Gln Lys Lys
        755                 760                 765

Glu Asp Ser Lys Thr Ser Thr Ser Val Thr Ser Val Asn Gln Ala Ser
    770                 775                 780

Thr Ser Arg Leu Glu Gly Leu Gln Ser Glu Asn His Arg Leu Arg Met
785                 790                 795                 800

Lys Ile Thr Glu Leu Asp Lys Asp Leu Glu Glu Val Thr Met Gln Leu
                805                 810                 815

Gln Asp Thr Pro Glu Lys Thr Thr Tyr Ile Lys Gln Asn His Tyr Gln
            820                 825                 830

Glu Leu Asn Asp Ile Leu Asn Leu Gly Asn Phe Thr Glu Ser Thr Asp
        835                 840                 845

Gly Gly Lys Ala Ile Leu Lys Asn His Leu Asp Gln Asn Pro Gln Leu
    850                 855                 860

Gln Trp Asn Thr Thr Glu Pro Ser Arg Thr Cys Lys Asp Pro Ile Glu
865                 870                 875                 880

Asp Ile Asn Ser Pro Glu His Ile Gln Arg Arg Leu Ser Leu Gln Leu
                885                 890                 895

Pro Ile Leu His His Ala Tyr Leu Pro Ser Ile Gly Gly Val Asp Ala
            900                 905                 910

Ser Cys Val Ser Pro Cys Val Ser Pro Thr Ala Ser Pro Arg His Arg
        915                 920                 925

His Val Pro Pro Ser Phe Arg Val Met Val Ser Gly Leu
    930                 935                 940


<210> SEQ ID NO 48
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Peptide

<400> SEQUENCE: 48

Pro Leu Tyr Ser Ile Leu Ser Ala Leu Thr Ile Leu Gly Met Ile Met
  1               5                  10                  15

Ala Ser Ala Phe Leu Phe Phe Asn Ile Lys Asn
             20                  25
```

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<210> SEQ ID NO 49
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Peptide

<400> SEQUENCE: 49

Leu Ile Ile Leu Gly Gly Met Leu Ser Tyr Ala Ser Ile Phe Leu Phe
1 5 10 15

Gly Leu Asp Gly Ser Phe Val Ser Glu Lys Thr
20 25

<210> SEQ ID NO 50
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Peptide

<400> SEQUENCE: 50

Cys Thr Val Arg Thr Trp Ile Leu Thr Val Gly Tyr Thr Thr Ala Phe
1 5 10 15

Gly Ala Met Phe Ala Lys Thr Trp Arg
20 25

<210> SEQ ID NO 51
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Peptide

<400> SEQUENCE: 51

Gln Lys Leu Leu Val Ile Val Gly Gly Met Leu Leu Ile Asp Leu Cys
1 5 10 15

Ile Leu Ile Cys Trp Gln
20

<210> SEQ ID NO 52
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Peptide

<400> SEQUENCE: 52

Met Thr Ile Trp Leu Gly Ile Val Tyr Ala Tyr Lys Gly Leu Leu Met
1 5 10 15

Leu Phe Gly Cys Phe Leu Ala Trp
20

<210> SEQ ID NO 53
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Peptide

<400> SEQUENCE: 53

Ala Leu Asn Asp Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly
1 5 10 15

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Ile Met Cys Ile Ile Gly Ala Ala Val
            20                  25


<210> SEQ ID NO 54
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Peptide

<400> SEQUENCE: 54

Ala Leu Asn Asp Ser Lys Tyr Ile Gly Met Ser Val Tyr Asn Val Gly
  1               5                  10                  15

Ile Met Cys Ile Ile Gly Ala Ala Val
            20                  25


<210> SEQ ID NO 55
<211> LENGTH: 844
<212> TYPE: PRT
<213> ORGANISM: Rattus Sp.

<400> SEQUENCE: 55

Met Gly Pro Gly Gly Pro Cys Thr Pro Val Gly Trp Pro Leu Pro Leu
  1               5                  10                  15

Leu Leu Val Met Ala Ala Gly Val Ala Pro Val Trp Ala Ser His Ser
            20                  25                  30

Pro His Leu Pro Arg Pro His Pro Arg Val Pro Pro His Pro Ser Ser
        35                  40                  45

Glu Arg Arg Ala Val Tyr Ile Gly Ala Leu Phe Pro Met Ser Gly Gly
    50                  55                  60

Trp Pro Gly Gly Gln Ala Cys Gln Pro Ala Val Glu Met Ala Leu Glu
65                  70                  75                  80

Asp Val Asn Ser Arg Arg Asp Ile Leu Pro Asp Tyr Glu Leu Lys Leu
                85                  90                  95

Ile His His Asp Ser Lys Cys Asp Pro Gly Gln Ala Thr Lys Tyr Leu
            100                 105                 110

Tyr Glu Leu Leu Tyr Asn Asp Pro Ile Lys Ile Ile Leu Met Pro Gly
        115                 120                 125

Cys Ser Ser Val Ser Thr Leu Val Ala Glu Ala Ala Arg Met Trp Asn
    130                 135                 140

Leu Ile Val Leu Ser Tyr Gly Ser Ser Ser Pro Ala Leu Ser Asn Arg
145                 150                 155                 160

Gln Arg Phe Pro Thr Phe Phe Arg Thr His Pro Ser Ala Thr Leu His
                165                 170                 175

Asn Pro Thr Arg Val Lys Leu Phe Glu Lys Trp Gly Trp Lys Lys Ile
            180                 185                 190

Ala Thr Ile Gln Gln Thr Thr Glu Val Phe Thr Ser Thr Leu Asp Asp
        195                 200                 205

Leu Glu Glu Arg Val Lys Glu Ala Gly Ile Glu Ile Thr Phe Arg Gln
    210                 215                 220

Ser Phe Phe Ser Asp Pro Ala Val Pro Val Lys Asn Leu Lys Arg Gln
225                 230                 235                 240

Asp Ala Arg Ile Ile Val Gly Leu Phe Tyr Glu Thr Glu Ala Arg Lys
                245                 250                 255

Val Phe Cys Glu Val Tyr Lys Glu Arg Leu Phe Gly Lys Lys Tyr Val
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            260                 265                 270

Trp Phe Leu Ile Gly Trp Tyr Ala Asp Asn Trp Phe Lys Thr Tyr Asp
        275                 280                 285

Pro Ser Ile Asn Cys Thr Val Glu Glu Met Thr Glu Ala Val Glu Gly
    290                 295                 300

His Ile Thr Thr Glu Ile Val Met Leu Asn Pro Ala Asn Thr Arg Ser
305                 310                 315                 320

Ile Ser Asn Met Thr Ser Gln Glu Phe Val Glu Lys Leu Thr Lys Arg
                325                 330                 335

Leu Lys Arg His Pro Glu Glu Thr Gly Gly Phe Gln Glu Ala Pro Leu
            340                 345                 350

Ala Tyr Asp Ala Ile Trp Ala Leu Ala Leu Ala Leu Asn Lys Thr Ser
        355                 360                 365

Gly Gly Gly Gly Arg Ser Gly Val Arg Leu Glu Asp Phe Asn Tyr Asn
    370                 375                 380

Asn Gln Thr Ile Thr Asp Gln Ile Tyr Arg Ala Met Asn Ser Ser Ser
385                 390                 395                 400

Phe Glu Gly Val Ser Gly His Val Val Phe Asp Ala Ser Gly Ser Arg
                405                 410                 415

Met Ala Trp Thr Leu Ile Glu Gln Leu Gln Gly Gly Ser Tyr Lys Lys
            420                 425                 430

Ile Gly Tyr Tyr Asp Ser Thr Lys Asp Asp Leu Ser Trp Ser Lys Thr
        435                 440                 445

Asp Lys Trp Ile Gly Gly Ser Pro Pro Ala Asp Gln Thr Leu Val Ile
    450                 455                 460

Lys Thr Phe Arg Phe Leu Ser Gln Lys Leu Phe Ile Ser Val Ser Val
465                 470                 475                 480

Leu Ser Ser Leu Gly Ile Val Leu Ala Val Val Cys Leu Ser Phe Asn
                485                 490                 495

Ile Tyr Asn Ser His Val Arg Tyr Ile Gln Asn Ser Gln Pro Asn Leu
            500                 505                 510

Asn Asn Leu Thr Ala Val Gly Cys Ser Leu Ala Leu Ala Ala Val Phe
        515                 520                 525

Pro Leu Gly Leu Asp Gly Tyr His Ile Gly Arg Ser Gln Phe Pro Phe
    530                 535                 540

Val Cys Gln Ala Arg Leu Trp Leu Leu Gly Leu Gly Phe Ser Leu Gly
545                 550                 555                 560

Tyr Gly Ser Met Phe Thr Lys Ile Trp Trp Val His Thr Val Phe Thr
                565                 570                 575

Lys Lys Glu Glu Lys Lys Glu Trp Arg Lys Thr Leu Glu Pro Trp Lys
            580                 585                 590

Leu Tyr Ala Thr Val Gly Leu Leu Val Gly Met Asp Val Leu Thr Leu
        595                 600                 605

Ala Ile Trp Gln Ile Val Asp Pro Leu His Arg Thr Ile Glu Thr Phe
    610                 615                 620

Ala Lys Glu Glu Pro Lys Glu Asp Ile Asp Val Ser Ile Leu Pro Gln
625                 630                 635                 640

Leu Glu His Cys Ser Ser Lys Lys Met Asn Thr Trp Leu Gly Ile Phe
                645                 650                 655

Tyr Gly Tyr Lys Gly Leu Leu Leu Leu Leu Gly Ile Phe Leu Ala Tyr
            660                 665                 670
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-continued

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Glu Thr Lys Ser Val Ser Thr Glu Lys Ile Asn Asp His Arg Ala Val
        675                 680                 685

Gly Met Ala Ile Tyr Asn Val Ala Val Leu Cys Leu Ile Thr Ala Pro
    690                 695                 700

Val Thr Met Ile Leu Ser Ser Gln Gln Asp Ala Ala Phe Ala Phe Ala
705                 710                 715                 720

Ser Leu Ala Ile Val Phe Ser Ser Tyr Ile Thr Leu Val Val Leu Phe
                725                 730                 735

Val Pro Lys Met Arg Arg Leu Ile Thr Arg Gly Glu Trp Gln Ser Glu
            740                 745                 750

Thr Gln Asp Thr Met Lys Thr Gly Ser Ser Thr Asn Asn Asn Glu Glu
        755                 760                 765

Glu Lys Ser Arg Leu Leu Glu Lys Glu Asn Arg Glu Leu Glu Lys Ile
    770                 775                 780

Ile Ala Glu Lys Glu Glu Arg Val Ser Glu Leu Arg His Gln Leu Gln
785                 790                 795                 800

Ser Arg Gln Gln Leu Arg Ser Arg Arg His Pro Pro Thr Pro Pro Asp
                805                 810                 815

Pro Ser Gly Gly Leu Pro Arg Gly Pro Ser Glu Pro Pro Asp Arg Leu
            820                 825                 830

Ser Cys Asp Gly Ser Arg Val His Leu Leu Tyr Lys
        835                 840
```

What is claimed is:

1. An isolated nucleic acid encoding a $GABA_BR2$ polypeptide.

2. The nucleic acid of claim 1, wherein the nucleic acid is DNA.

3. The DNA of claim 2, wherein the DNA is cDNA.

4. The DNA of claim 2, wherein the DNA is genomic DNA.

5. The nucleic acid of claim 1, wherein the nucleic acid is RNA.

6. The nucleic acid of claim 1, wherein the nucleic acid encodes a mammalian $GABA_BR2$ polypeptide.

7. The nucleic acid of claim 1, wherein the nucleic acid encodes a rat $GABA_BR2$ polypeptide.

8. The nucleic acid of claim 1, wherein the nucleic acid encodes a human $GABA_BR2$ polypeptide.

9. The nucleic acid of claim 6, wherein the nucleic acid encodes a polypeptide characterized by an amino acid sequence in the transmembrane regions which has an identity of 90% or higher to the amino acid sequence in the transmembrane regions of the human $GABA_BR2$ polypeptide shown in FIGS. 5A-5D.

10. The nucleic acid of claim 6, wherein the nucleic acid encodes a mammalian $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as does the $GABA_BR2$ polypeptide encoded by the plasmid BO-55 (ATCC Accession No. 209104).

11. The nucleic acid of claim 7, wherein the nucleic acid encodes a rat $GABA_BR2$ polypeptide which has an amino acid sequence encoded by the plasmid BO-55 (ATCC Accession No. 209104).

12. The nucleic acid of claim 7, wherein the nucleic acid encodes a rat $GABA_BR2$ polypeptide having substantially the same amino acid sequence as the amino acid sequence shown in FIGS. 4A-4D (SEQ ID NO:4).

13. The nucleic acid of claim 7, wherein the rat $GABA_BR2$ polypeptide has an amino acid sequence which comprises the amino acid sequence shown in FIGS. 4A-4D (SEQ ID NO:4).

14. The nucleic acid of claim 6, wherein the nucleic acid encodes a mammalian $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as does the $GABA_BR2$ polypeptide encoded by the plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

15. The nucleic acid of claim 8, wherein the human $GABA_BR2$ polypeptide comprises an amino acid sequence substantially the same as the amino acid sequence encoded by plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

16. The nucleic acid of claim 8, wherein the human $GABA_BR2$ polypeptide comprises an amino acid sequence substantially the same as the amino acid sequence in FIGS. 23A-23D (SEQ ID NO:47).

17. The nucleic acid of claim 8, wherein the human $GABA_BR2$ polypeptide has an amino acid sequence which comprises the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

18. A purified $GABA_BR2$ protein.

19. A vector comprising the nucleic acid of claim 1.

20. A vector comprising the nucleic acid of claim 8.

21. A vector of claim 19 adapted for expression in a bacterial cell which comprises the regulatory elements necessary for expression of the nucleic acid in the bacterial cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

22. A vector of claim 19 adapted for expression in an amphibian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the amphibian cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

23. A vector of claim 19 adapted for expression in a yeast cell which comprises the regulatory elements necessary for expression of the nucleic acid in the yeast cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

24. A vector of claim 19 adapted for expression in an insect cell which comprises the regulatory elements necessary for expression of the nucleic acid in the insect cell operatively linked to the nucleic acid encoding the $GABA_BR2$ polypeptide so as to permit expression thereof.

25. A vector of claim 24 which is a baculovirus.

26. A vector of claim 19 adapted for expression in a mammalian cell which comprises the regulatory elements necessary for expression of the nucleic acid in the mammalian cell operatively linked to the nucleic acid encoding a $GABA_BR2$ polypeptide so as to permit expression thereof.

27. A vector of claim 19 wherein the vector is a plasmid.

28. The plasmid of claim 27 designated BO-55 (ATCC Accession No. 209104).

29. The plasmid of claim 27 designated pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

30. A method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within one of the two strands of the nucleic acid encoding the $GABA_BR2$ polypeptide contained in plasmid BO-55, and detecting hybridization of the probe to the nucleic acid.

31. A method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the nucleic acid sequence shown in FIGS. 22A-22D (SEQ ID NO:46) or (b) the reverse complement to the nucleic acid sequence shown in FIGS. 22A-22D (SEQ ID NO:46), and detecting hybridization of the probe to the nucleic acid.

32. A method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within one of the two strands of the nucleic acid encoding the $GABA_BR2$ polypeptide contained in plasmid pEXJT3T7-hGABAB2, and detecting hybridization of the probe to the nucleic acid.

33. A method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising at least 15 nucleotides, which probe specifically hybridizes with the nucleic acid encoding the $GABA_BR2$ polypeptide, wherein the probe has a unique sequence, which sequence is present within (a) the nucleic acid sequence shown in FIGS. 3A-3D (SEQ ID NO:3) or (b) the reverse complement to the nucleic acid sequence shown in FIGS. 3A-3D (SEQ ID NO:3), and detecting hybridization of the probe to the nucleic acid.

34. The method of any one of claims 30 to 33, wherein the nucleic acid is DNA.

35. The method of any one of claims 30 to 33, wherein the nucleic acid is RNA.

36. The method of any one of claims 30 to 33, wherein the probe comprises at least 15 nucleotides complementary to a unique segment of the sequence of the nucleic acid molecule encoding the $GABA_BR2$ polypeptide.

37. A method of detecting a nucleic acid encoding a $GABA_BR2$ polypeptide, which comprises contacting the nucleic acid with a probe comprising a nucleic acid of at least 15 nucleotides which is complementary to the antisense sequence of a unique segment of the sequence of the nucleic acid encoding the $GABA_BR2$ polypeptide, and detecting hybridization of the probe to the nucleic acid.

38. A method of inhibiting translation of mRNA encoding a $GABA_BR2$ polypeptide which comprises contacting such mRNA with an antisense oligonucleotide having a sequence capable of specifically hybridizing to the mRNA of claim 5, so as to prevent translation of the mRNA.

39. A method of inhibiting translation of mRNA encoding a $GABA_BR2$ polypeptide which comprises contacting such mRNA with an antisense oligonucleotide having a sequence capable of specifically hybridizing to the genomic DNA of claim 4.

40. The method of claim 38 or 39, wherein the oligonucleotide comprises chemically modified nucleotides or nucleotide analogues.

41. An isolated antibody capable of binding to a $GABA_BR2$ polypeptide encoded by the nucleic acid of claim 1.

42. The antibody of claim 41, wherein the $GABA_BR2$ polypeptide is a human $GABA_BR2$ polypeptide.

43. An antibody capable of competitively inhibiting the binding of the antibody of claim 41 to a $GABA_BR2$ polypeptide.

44. An antibody of claim 41, wherein the antibody is a monoclonal antibody.

45. A monoclonal antibody of claim 44 directed to an epitope of a $GABA_BR2$ polypeptide present on the surface of a $GABA_BR2$ polypeptide expressing cell.

46. A method of claim 38 or 39, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

47. A method of claim 46, wherein the substance which inactivates mRNA is a ribozyme.

48. A pharmaceutical composition which comprises an amount of the antibody of claim 41 effective to block binding of a ligand to the $GABA_BR2$ polypeptide and a pharmaceutically acceptable carrier.

49. A transgenic, nonhuman mammal expressing DNA encoding a $GABA_BR2$ polypeptide of claim 1.

50. A transgenic, nonhuman mammal comprising a homologous recombination knockout of the native $GABA_BR2$ polypeptide.

51. A transgenic, nonhuman mammal whose genome comprises antisense DNA complementary to DNA encoding a $GABA_BR2$ polypeptide of claim 1 so placed as to be transcribed into antisense mRNA which is complementary to mRNA encoding such $GABA_BR2$ polypeptide and which hybridizes to such mRNA encoding such $GABA_BR2$ polypeptide, thereby reducing its translation.

52. The transgenic, nonhuman mammal of claim 49 or 50, wherein the DNA encoding the $GABA_BR2$ polypeptide additionally comprises an inducible promoter.

53. The transgenic, nonhuman mammal of claim 49 or 50, wherein the DNA encoding the $GABA_BR2$ polypeptide additionally comprises tissue specific regulatory elements.

54. A transgenic, nonhuman mammal of any one of claims 49, 50 or 51, wherein the transgenic, nonhuman mammal is a mouse.

55. A method of detecting the presence of a $GABA_BR2$ polypeptide on the surface of a cell which comprises contacting the cell with the antibody of claim 41 under conditions permitting binding of the antibody to the polypeptide, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a $GABA_BR2$ polypeptide on the surface of the cell.

56. A method of preparing the purified $GABA_BR2$ polypeptide of claim 18 which comprises:

a. inducing cells to express a $GABA_BR2$ polypeptide;

b. recovering the polypeptide so expressed from the induced cells; and

c. purifying the polypeptide so recovered.

57. A method of preparing the purified $GABA_BR2$ polypeptide of claim 18 which comprises:

a. inserting a nucleic acid encoding the $GABA_BR2$ polypeptide into a suitable vector;

b. introducing the resulting vector in a suitable host cell;

c. placing the resulting cell in suitable condition permitting the production of the $GABA_BR2$ polypeptide;

d. recovering the polypeptide produced by the resulting cell; and

e. isolating or purifying the polypeptide so recovered.

58. A $GABA_BR1/R2$ receptor comprising two polypeptides, one of which is a $GABA_BR2$ polypeptide and another of which is a $GABA_BR1$ polypeptide.

59. A method of forming a $GABA_BR1/R2$ receptor which comprises inducing cells to express both a $GABA_BR1$ polypeptide and a $GABA_BR2$ polypeptide.

60. An antibody capable of binding to a $GABA_BR1/R2$ receptor, wherein the $GABA_BR2$ polypeptide is encoded by the nucleic acid of claim 1.

61. The antibody of claim 60, wherein the $GABA_BR2$ polypeptide is a human $GABA_BR2$ polypeptide.

62. An antibody capable of competitively inhibiting the binding of the antibody of claim 60 to a $GABA_BR1/R2$ receptor.

63. An antibody of claim 60, wherein the antibody is a monoclonal antibody.

64. A monoclonal antibody of claim 63 directed to an epitope of a $GABA_BR1/R2$ receptor present on the surface of a $GABA_BR1/R2$ polypeptide expressing cell.

65. A pharmaceutical composition which comprises an amount of the antibody of claim 60 effective to block binding of a ligand to the $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

66. A transgenic, nonhuman mammal expressing a $GABA_BR1/R2$ receptor, which is not naturally expressed by the mammal.

67. A transgenic, nonhuman mammal comprising a homologous recombination knockout of the native $GABA_BR1/R2$ receptor.

68. A transgenic, nonhuman mammal of claim 66 or 67, wherein the transgenic nonhuman mammal is a mouse.

69. A method of detecting the presence of a $GABA_BR1/R2$ receptor on the surface of a cell which comprises contacting the cell with the antibody of claim 60 under conditions permitting binding of the antibody to the receptor, detecting the presence of the antibody bound to the cell, and thereby detecting the presence of a $GABA_BR1/R2$ receptor on the surface of the cell.

70. A method of determining the physiological effects of varying levels of activity of $GABA_BR1/R2$ receptors which comprises producing a transgenic nonhuman mammal of claim 66 whose levels of $GABA_BR1/R2$ receptor activity vary due to the presence of an inducible promoter which regulates $GABA_BR1/R2$ receptor expression.

71. A method of determining the physiological effects of varying levels of activity of $GABA_BR1/R2$ receptors which comprises producing a panel of transgenic nonhuman mammals of claim 66, each expressing a different amount of $GABA_BR1/R2$ receptor.

72. A method for identifying an antagonist capable of alleviating an abnormality, by decreasing the activity of a $GABA_BR1/R2$ receptor comprising administering a compound to the transgenic nonhuman mammal of claim 66 or 68, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as the antagonist.

73. An antagonist identified by the method of claim 72.

74. A pharmaceutical composition comprising an antagonist of claim 73 and a pharmaceutically acceptable carrier.

75. A method of treating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of a $GABA_BR1/R2$ receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 74, thereby treating the abnormality.

76. A method for identifying an agonist capable of alleviating an abnormality, by increasing the activity of a $GABA_BR1/R2$ receptor comprising administering a compound to the transgenic nonhuman mammal of claim 66 or 68, and determining whether the compound alleviates the physical and behavioral abnormalities displayed by the transgenic, nonhuman mammal, the alleviation of the abnormality identifying the compound as the agonist.

77. An agonist identified by the method of claim 76.

78. A pharmaceutical composition comprising an agonist of claim 76 and a pharmaceutically acceptable carrier.

79. A method for treating an abnormality in a subject wherein the abnormality is alleviated by increasing the activity of a $GABA_BR$ 1/R2 receptor which comprises administering to a subject an effective amount of the pharmaceutical composition of claim 78, thereby treating the abnormality.

80. A cell which expresses on its surface a mammalian $GABA_BR1/R2$ receptor that is not naturally expressed on the surface of such cell.

81. A cell of claim 80, wherein the mammalian $GABA_BR1/R2$ receptor comprises two polypeptides, one of which is a $GABA_BR2$ polypeptide and another of which is a $GABA_BR1$ polypeptide.

82. A process for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/$

R2 receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor.

83. A process for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises contacting a membrane fraction from a cell extract of cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_nR1/R2$ receptor, with the compound under conditions suitable for binding, and detecting specific binding of the chemical compound to the $GABA_BR1/R2$ receptor.

84. The process of claim 82 or **83**, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

85. The process of claim 82 or **83**, wherein the $GABA_nR1/R2$ receptor comprises a $GABA_nR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

86. The process of claim 82 or **83**, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same sequence as the amino acid sequence shown in FIGS. **23A-23D** (SEQ ID NO:47).

87. The process of claim 82 or **83**, wherein the $GABA_nR1/R2$ receptor comprises a $GABA_nR2$ polypeptide which has the amino acid sequence shown in FIGS. **23A-23D** (SEQ ID NO:47).

88. The process of claims **82** or **83**, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

89. The process of claim 82 or **83**, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as the sequence shown in FIGS. **23A-23D** (SEQ ID NO:47).

90. The process of claim 82 or **83**, wherein the $GABA_nR1/R2$ receptor comprises a $GABA_nR2$ polypeptide which has the sequence shown in FIGS. **23A-23D** (SEQ ID NO:47).

91. The process of claim 89, wherein the compound is not previously known to bind to a $GABA_BR1/R2$ receptor.

92. A compound identified by the process of claim 91.

93. A process of claim 89, wherein the cell is an insect cell.

94. A process of claim 89, wherein the cell is a mammalian cell.

95. A process of claim 94, wherein the cell is nonneuronal in origin.

96. A process of claim 95, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk−) cell.

97. A process of claim 94, wherein the compound is not previously known to bind to a $GABA_BR1/R2$ receptor.

98. A compound identified by the process of claim 97.

99. A process involving competitive binding for identifying a chemical compound which specifically binds to a $GABA_BR1/R2$ receptor which comprises separately contacting cells expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the $GABA_nR1/R2$ receptor, a decrease in the binding of the second chemical compound to the $GABA_BR1/R2$ receptor in the presence of the chemical compound indicating that the chemical compound binds to the $GABA_BR1/R2$ receptor.

100. A process involving competitive binding for identifying a chemical compound which specifically binds to a human $GABA_BR1/R2$ receptor which comprises separately contacting a membrane fraction from a cell extract of cells expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical compound known to bind to the receptor, and with only the second chemical compound, under conditions suitable for binding of both compounds, and detecting specific binding of the chemical compound to the $GABA_nR1/R2$ receptor, a decrease in the binding of the second chemical compound to the $GABA_BR1/R2$ receptor in the presence of the chemical compound indicating that the chemical compound binds to the $GABA_BR1/R2$ receptor.

101. A process of claim 99 or **100**, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

102. The process of claim 101, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_nR2$ polypeptide which has substantially the same amino acid sequence as that encoded by plasmid BO-55 (ATCC Accession No. 209104).

103. The process of claim 99 or **100**, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in FIGS. **23A-23D** (SEQ ID NO:47).

104. The process of claim 99 or **100**, wherein the $GABA_nR1/R2$ receptor comprises a $GABA_nR2$ polypeptide which has the amino acid sequence shown in FIGS. **23A-23D** (Seq. ID NO:47).

105. The process of claim 99 or **100**, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by plasmid pEXJT3T7-hGABAB2 (ATCC Accession No.203515).

106. The process of claim 99 or **100**, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as the sequence shown in FIGS. **23A-23D** (SEQ ID NO:47).

107. The process of claim 99 or **100**, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence shown in FIGS. **23A-23D** (SEQ ID NO:47).

108. The process of claim 107, wherein the cell is an insect cell.

109. The process of claim 107, wherein the cell is a mammalian cell.

110. The process of claim 109, wherein the cell is nonneuronal in origin.

111. The process of claim 110, wherein the nonneuronal cell is a COS-7 cell, 293 human embryonic kidney cell, a CHO cell, a NIH-3T3 cell a mouse Y1 cell or LM(tk−) cell.

112. The process of claim 109, wherein the compound is not previously known to bind to a $GABA_BR1/R2$ receptor.

113. A compound identified by the process of claim 112.

114. A method of screening a plurality of chemical compounds not known to bind to a $GABA_BR1/R2$ receptor to identify a compound which specifically binds to the $GABA_BR1/R2$ receptor, which comprises

(a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with a compound known to bind specifically to the $GABA_BR1/R2$ receptor;

(b) contacting the same cells as in step (a) with the plurality of compounds not known to bind specifically to the $GABA_BR1/R2$ receptor, under conditions permitting binding of compounds known to bind the $GABA_BR1/R2$ receptor;

(c) determining whether the binding of the compound known to bind specifically to the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of the compounds, relative to the binding of the compound in the absence of the plurality of compounds, and if the binding is reduced;

(d) separately determining the extent of binding to the $GABA_BR1/R2$ receptor of each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the $GABA_BR1/R2$ receptor.

115. A method of screening a plurality of chemical compounds not known to bind to a $GABA_BR1/R2$ receptor to identify a compound which specifically binds to the $GABA_BR1/R2$ receptor, which comprises

(a) contacting a membrane fraction extract from cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with a compound known to bind specifically to the $GABA_BR1/R2$ receptor;

(b) contacting the same membrane fraction as in step (a) with the plurality of compounds not known to bind specifically to the $GABA_BR1/R2$ receptor, under conditions permitting binding of compounds known to bind the $GABA_BR1/R2$ receptor;

(c) determining whether the binding of the compound known to bind specifically to the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of compounds, relative to the binding of the compound in the absence of the plurality of compounds, and if the binding is reduced;

(d) separately determining the extent of binding to the $GABA_BR1/R2$ receptor of each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which specifically binds to the $GABA_BR1/R2$ receptor.

116. A method of claim 114 or **115**, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

117. A method of either of claim 114 or **115**, wherein the cell is a mammalian cell.

118. A method of claim 117, wherein the mammalian cell is non-neuronal in origin.

119. The method of claim 118, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk−) cell, a CHO cell, a mouse Y1 cell or an NIH-3T3 cell.

120. A process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor agonist which comprises contacting cells with the compound under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting an increase in $GABA_BR1/R2$ receptor activity, so as to thereby determine whether the compound is a $GABA_BR1/R2$ receptor agonist.

121. A process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor antagonist which comprises contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the compound in the presence of a known $GABA_BR1/R2$ receptor agonist, under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting a decrease in $GABA_BR1/R2$ receptor activity, so as to thereby determine whether the compound is a $GABA_BR1/R2$ receptor antagonist.

122. A process of claim 120 or **121**, wherein the cells additionally express nucleic acid encoding GIRK1 and GIRK4.

123. A process of any one of claims **120**, **121**, or **122**, wherein the $GABA_BR2$ receptor is a mammalian $GABA_BR2$ receptor.

124. A pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor agonist determined to be an agonist by the process of claim 120 effective to increase activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

125. A pharmaceutical composition of claim 124, wherein the $GABA_BR1/R2$ receptor agonist was not previously known.

126. A pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor antagonist determined to be an antagonist the process of claim 121 effective to reduce activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

127. A pharmaceutical composition of claim 126, wherein the $GABA_BR1/R2$ receptor antagonist was not previously known.

128. A process for determining whether a chemical compound activates a $GABA_BR1/R2$ receptor, which comprises contacting cells producing a second messenger response and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the chemical compound under conditions suitable for activation of the $GABA_BR1/R2$ receptor, and measuring the second messenger response in the presence and in the absence of the chemical compound, a change in the second messenger response in the presence of the chemical compound indicating that the compound activates the $GABA_BR1/R2$ receptor.

129. The process of claim 128, wherein the second messenger response comprises potassium channel activation and the change in second messenger is an increase in the level of potassium current.

130. A process for determining whether a chemical compound inhibits activation of a $GABA_BR1/R2$ receptor, which comprises separately contacting cells producing a second messenger response and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with both the chemical compound and a second chemical compound known to activate the $GABA_BR1/R2$ receptor, and with only

the second chemical compound, under conditions suitable for activation of the $GABA_BR1/R2$ receptor, and measuring the second messenger response in the presence of only the second chemical compound and in the presence of both the second chemical compound and the chemical compound, a smaller change in the second messenger response in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound indicating that the chemical compound inhibits activation of the $GABA_BR1/R2$ receptor.

131. The process of claim 130, wherein the second messenger response comprises potassium channel activation and the change in second messenger response is a smaller increase in the level of inward potassium current in the presence of both the chemical compound and the second chemical compound than in the presence of only the second chemical compound.

132. A process of any one of claims **128**, **129**, **130** or **131**, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

133. The process of claim 132, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

134. The process of claim 132, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 4A-4D (SEQ ID NO:4).

135. The process of claim 132, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 23A-23D (SEQ ID NO:47).

136. The process of claim 132, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has the sequence, shown in FIGS. 23A-23D (SEQ ID NO:47).

137. The process of claim 132, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

138. The process of any one of claims **128-131**, wherein the cell is an insect cell.

139. The process of any one of claims **128-131**, wherein the cell is a mammalian cell.

140. The process of claim 139, wherein the mammalian cell is nonneuronal in origin.

141. The process of claim 140, wherein the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk−) cell.

142. The process of claim 139, wherein the compound was not previously known to activate or inhibit a $GABA_BR1/R2$ receptor.

143. A compound determined by the process of claim 142.

144. A pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor agonist determined by the process of claim 128 or **129** effective to increase activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

145. A pharmaceutical composition of claim 144, wherein the $GABA_BR1/R2$ receptor agonist was not previously known.

146. A pharmaceutical composition which comprises an amount of a $GABA_BR1/R2$ receptor antagonist determined by the process of claim 130 or **131** effective to reduce activity of a $GABA_BR1/R2$ receptor and a pharmaceutically acceptable carrier.

147. A pharmaceutical composition of claim 146, wherein the $GABA_BR1/R2$ receptor antagonist was not previously known.

148. A method of screening a plurality of chemical compounds not known to activate a $GABA_BR$ 1/R2 receptor to identify a compound which activates the $GABA_BR1/R2$ receptor which comprises:

(a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the plurality of compounds not known to activate the $GABA_BR1/R2$ receptor, under conditions permitting activation of the $GABA_BR1/R2$ receptor;

(b) determining whether the activity of the $GABA_BR1/R2$ receptor is increased in the presence of the compounds, and if it is increased;

(c) separately determining whether the activation of the $GABA_BR1/R2$ receptor is increased by each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such plurality of compounds which activates the $GABA_BR1/R2$ receptor.

149. The process of claim 148, wherein the cells express nucleic acid encoding GIRK1 and GIRK4.

150. A method of claim 148 or **149**, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

151. A method of screening a plurality of chemical compounds not known to inhibit the activation of a $GABA_BR1/R2$ receptor to identify a compound which inhibits the activation of the $GABA_BR1/R2$ receptor, which comprises:

(a) contacting cells containing nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, with the plurality of compounds in the presence of a known $GABA_BR1/R2$ receptor agonist, under conditions permitting activation of the $GABA_BR1/R2$ receptor;

(b) determining whether the activation of the $GABA_BR1/R2$ receptor is reduced in the presence of the plurality of compounds, relative to the activation of the $GABA_BR1/R2$ receptor in the absence of the plurality of compounds, and if it is reduced;

(c) separately determining the inhibition of activation of the $GABA_BR1/R2$ receptor for each compound included in the plurality of compounds, so as to thereby identify the compound or compounds present in such a plurality of compounds which inhibits the activation of the $GABA_BR1/R2$ receptor.

152. The process of claim 151, wherein the cells express nucleic acid encoding GIRK1 and GIRK4.

153. A method of claim 151 or **152**, wherein the $GABA_BR1/R2$ receptor is a mammalian $GABA_BR1/R2$ receptor.

154. A method of any one of claims **148**, **149**, **151**, or **152**, wherein the cell is a mammalian cell.

155. A method of claim 154, wherein the mammalian cell is non-neuronal in origin.

156. The method of claim 155, wherein the non-neuronal cell is a COS-7 cell, a 293 human embryonic kidney cell, a LM(tk−) cell or an NIH-3T3 cell.

157. A pharmaceutical composition comprising a compound identified by the method of claim 148 or **149**, effective to increase $GABA_BR1/R2$ receptor activity and a pharmaceutically acceptable carrier.

158. A pharmaceutical composition comprising a compound identified by the method of claim 151 or **152**, effective to decrease $GABA_BR1/R2$ receptor activity and a pharmaceutically acceptable carrier.

159. A process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor agonist, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR1/R2$ receptor, separately contacting the membrane fraction with both the chemical compound and GTPYS, and with only GTPYS, under conditions permitting the activation of the $GABA_BR1/R2$ receptor, and detecting GTPYS binding to the membrane fraction, an increase in GTPYS binding in the presence of the compound indicating that the chemical compound activates the $GABA_BR1/R2$ receptor.

160. A process for determining whether a chemical compound is a $GABA_BR1/R2$ receptor antagonist, which comprises preparing a membrane fraction from cells which comprise nucleic acid encoding and expressing on their cell surface the $GABA_BR1/R2$ receptor, wherein such cells do not normally express the $GABA_BR$ 1/R2 receptor, separately contacting the membrane fraction with the chemical compound, GTPYS and a second chemical compound known to activate the $GABA_BR1/R2$ receptor, with GTPYS and only the second compound, and with GTPYS alone, under conditions permitting the activation of the $GABA_BR1/R2$ receptor, detecting GTPYS binding to each membrane fraction, and comparing the increase in GTPYS binding in the presence of the compound and the second compound relative to the binding of GTPYS alone, to the increase in GTPYS binding in the presence of the second chemical compound known to activate the $GABA_BR1/R2$ receptor relative to the binding of GTPYS alone, a smaller increase in GTPYS binding in the presence of the compound and the second compound indicating that the compound is a $GABA_BR1/R2$ receptor antagonist.

161. A process of claim 159 or **160**, wherein the $GABA_BR2$ receptor is a mammalian $GABA_BR2$ receptor.

162. The process of claim 161, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid BO-55 (ATCC Accession No. 209104).

163. The process of claim 162, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 4A-4D (SEQ ID NO:4).

164. The process of claim 161, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that encoded by the plasmid pEXJT3T7-hGABAB2 (ATCC Accession No. 203515).

165. The process of claim 161, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_BR2$ polypeptide which has substantially the same amino acid sequence as that shown in FIGS. 23A-23D (SEQ ID NO:47).

166. The process of claim 161, wherein the $GABA_BR1/R2$ receptor comprises a $GABA_nR2$ polypeptide which has the sequence shown in FIGS. 23A-23D (SEQ ID NO:47).

167. The process of claim 159 or **160**, wherein the cell is an insect cell.

168. The process of claim 159 or **160**, wherein the cell is a mammalian cell.

169. The process of claim 168, wherein the mammalian cell is nonneuronal in origin.

170. The process of claim 169, wherein the nonneuronal cell is a COS-7 cell, CHO cell, 293 human embryonic kidney cell, NIH-3T3 cell or LM(tk−) cell.

171. The process of claim 170, wherein the compound was not previously known to be an agonist or antagonist of a $GABA_BR1/R2$ receptor.

172. A compound determined to be an agonist or antagonist of a $GABA_BR1/R2$ receptor by the process of claim 171.

173. A method of treating spasticity in a subject which comprises administering to the subject an amount of a compound which is an agonist of a $GABA_nR1/R2$ receptor effective to treat spasticity in the subject.

174. A method of treating asthma in a subject which comprises administering to the subject an amount of a compound which is a $GABA_nR1/R2$ receptor agonist effective to treat asthma in the subject.

175. A method of treating incontinence in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to treat incontinence in the subject.

176. A method of decreasing nociception in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to decrease nociception in the subject.

177. A use of a $GABA_BR2$ agonist as an antitussive agent which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective as an antitussive agent in the subject.

178. A method of treating drug addiction in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor agonist effective to treat drug addiction in the subject.

179. A method of treating Alzheimer's disease in a subject which comprises administering to the subject an amount of a compound which is a $GABA_BR1/R2$ receptor antagonist effective to treat Alzheimer's disease in the subject.

182. A process for making a composition of matter which specifically binds to a $GABA_nR1/R2$ receptor which comprises identifying a chemical compound using the process of any of claims, **82**, **83**, **99**, **100**, **114** or **115** and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

183. A process for making a composition of matter which specifically binds to a $GABA_BR1/R2$ receptor which comprises identifying a chemical compound using the process of any of claims **120**, **128**, or **148** and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

184. A process for making a composition of matter which specifically binds to a $GABA_BR1/R2$ receptor which comprises identifying a chemical compound using the process of

any of claims **121, 130,** or **151** and then synthesizing the chemical compound or a novel structural and functional analog or homolog thereof.

185. The process of any of claims **182, 183,** or **184,** wherein the $GABA_BR1/R2$ receptor is a human $GABA_BR1/R2$ receptor.

186. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims **82, 83, 99, 100, 114** or **115** or a novel structural and functional analog or homolog thereof.

187. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims **120, 128,** or **148** or a novel structural and functional analog or homolog thereof.

188. A process for preparing a pharmaceutical composition which comprises admixing a pharmaceutically acceptable carrier and a pharmaceutically acceptable amount of a chemical compound identified by the process of any of claims **121, 130,** or **151** or a novel structural and functional analog or homolog thereof.

189. The process of any of claims **186, 187,** or **188,** wherein the $GABA_BR1/R2$ receptor is a human $GABA_BR1/R2$. receptor.

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